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Title	Etiology, epidemiology and control of the bacterial diseases, potato blackleg and soft rot, and chemical control of certain fungal diseases of potato tubers : with a short section on the bacteriology and ecology of the bacterium Erwinia herbicola (Lohnis) dye
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ETIOLOGY, EPIDEMIOLOGY AND CONTROL OF THE BACTERIAL DISEASES, POTATO BLACKLEG AND SOFT ROT, AND CHEMICAL CONTROL OF CERTAIN FUNGAL DISEASES OF POTATO TUBERS

(With a short section on the bacteriology and ecology of the bacterium Erwinia herbicola (Lohnis) Dye)

Presentation for the Degree of

Doctor of Science

by

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Frontispiece - Potato Blackleg Disease

ABSTRACT

The first part of the presentation begins by summarising early work on potato blackleg and soft rot starting with basic studies on the properties and bacteriological characteristics of the causal organisms (Erwinia carotovora and atroseptica), the nature of the diseases they bring about, and the apparent lack of survival of the bacteria in soils. This is followed by an outline of the production of "pathogen free" potato stocks by raising plants from stem cuttings, and their release into commerce in 1970. The third stage of the investigations is concerned with how these stocks may become infected by soft rot Erwinia bacteria through mechanical, insect, and airborne transmission (as an atmospheric aerosol). Finally, the most recent studies focus on the occurrence of the pathogens in association with roots of weeds and crop plants, and their presence in surface waters. Suggestions are made about how the results of the research point to practical methods which could be used by growers and others to minimise contamination of "pathogen-free" stocks. Additionally, suggestions are made regarding further research and development ultimately aiming to maintain and improve health of certified potato stocks.

The second part of the presentation deals with studies on chemical control of various potato tuber diseases, especially gangrene (Phoma exigua var. foveata), dry rot (Fusarium solani var. caeruleum) and skin spot (Polyscytalum pustulans). The activity of mercury compounds was studied at first but because of high toxicity and other undesirable properties, replacements were sought, first through organo-tin compounds, and then by use of antifungal fumigants. The discovery of the fumigant 2-aminobutane is described, and the technological developments to bring it into commercial use are explained. Fumigation is now extensively used in Scotland, especially to control gangrene and skin spot in high grade seed stocks.

Finally there is a brief account of studies on the ecology and taxonomy of certain yellow pigmented bacteria commonly associated with plant material - namely Erwinia herbicola.

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PREFACE

Potato diseases have been the concern of the Department of Agriculture and Fisheries for Scotland for many years. This arises from the Department's responsibility for the Scottish Potato Classification Scheme (formerly the Certification Scheme) and from the need to provide for disease control in relation to the potato export trade. The Classification Scheme began in 1918, the main aim being to control wart disease, caused by the fungus *Synchytrium endobioticum*.

Other diseases were first considered in the Classification Scheme as long ago as 1932, including severe virus diseases and blackleg. During the war of 1939-45, the importance of preventing losses caused by tuber diseases was recognised as an important contribution to safeguarding the nations food, and considerable efforts were made to understand and control tuber rots, especially dry rot (*Fusarium solani* var. *coeruleum*), because at that time it was one of the most prevalent diseases. Control of blight (*Phytophthora infestans*) had already been achieved, largely by spraying the crop with copper preparations (such as Bordeaux mixture), while control of dry rot was largely obtained by reducing tuber damage and by application of a dust containing tetrachloronitrobenzene (tecnazene, TCNB). The tuber skin disease, skin spot (*Oospora* (*Polyscytalum*) *pustulans*) was also common and serious in susceptible varieties, but the only known means of satisfactory control was by dipping in solutions of mercury compounds; this treatment also controlled dry rot. Meanwhile the

disease gangrene, (Phoma exigua var. foveata) had been described as long ago as 1936 (Alcock and Foister, 1936), but it rose to great importance in the early 1950s, for reasons which are not clearly understood. Nowadays, gangrene is probably the most important rot disease of potato; it is certainly one of the most common causes of complaint. The bacterial diseases blackleg and soft rot caused by species of Erwinia have been prevalent in Scotland for many years, but their occurrence always proved sporadic, with some varieties being particularly prone to the disease, while in some years the diseases were much more common than in others, but were always present to some degree. Before the 1950s no systematic attempt had been made to understand their epidemiology, and the nature and ecology of the pathogens, with a view to better control.

The diseases gangrene, skin spot, blackleg and soft rot often became manifest only after seed had been received by the purchaser, farmers and merchants referred to them as 'latent' diseases, that is to say undetectable by the producer but observed later by the purchaser. By early 1960 the position had become very serious, and various persons and organisations became involved in their study. The commercial impact of the diseases was discussed and summarised by Hay (1969).

DAFS Agricultural Scientific Services has been studying potato blackleg, soft rot, gangrene, and skin spot for more than 30 years. As regards the two latter diseases, much of the work was and indeed still remains devoted to the study of chemical control, whereas with blackleg and soft rot, no chemical treatment has yet been found which

gives satisfactory disease control, so that efforts have been concentrated on disease etiology and epidemiology.

Following the General Introduction, blackleg and soft rot are dealt with in Part 1 of this presentation, fungal diseases and their control in Part 2.

Finally, Part 3 briefly discusses the identity of certain rod-shaped, gram-negative, yellow-pigmented, fermentative, motile bacteria which are commonly found in association with plant material, sometimes with animals (including man), and sometimes in the general environment. Not infrequently these have been wrongly identified as the cause of various plant diseases, and opportunity was taken to study these organisms in the course of comparative bacteriological studies on all species of Erwinia undertaken at this laboratory.

ETIOLOGY, EPIDEMIOLOGY AND CONTROL OF POTATO BLACKLEG AND BACTERIAL SOFT ROT DISEASES, AND CHEMICAL CONTROL OF FUNGAL DISEASES OF POTATO TUBERS

GENERAL INTRODUCTION

In Scotland the bacterial diseases blackleg and tuber soft rot are, in the case of blackleg, caused by the bacterium Erwinia carotovora var. atroseptica (Van Hall) Dye (subsequently referred to as Erwinia atroseptica), and in the case of tuber soft rot by this organism, and also by Erwinia carotovora var. carotovora (Jones) Bergey et al. (subsequently referred to as Erwinia carotovora). When detailed investigations began by myself in 1955, it was known that these were very likely to be the causal organisms, but even so the identifications were based on very few isolates (Noble and Marshall, 1952). The two diseases were reasonably well known in Scotland as far as symptomatology and pathology are concerned - indeed they had been since official potato inspections began under the Classification Scheme. But methods of isolating and characterising the bacteria were poor and it was very uncertain whether or not the Erwinias were primarily soil-borne, that is to say part of the autochthonous bacterial flora of the soil, or tuber borne. The best resume available in the 1950s regarding the nature and cause of these diseases in Britain can be found in the book "The Potato in Health and Disease" by Whitehead, MacIntosh and Findlay, 3rd edition, Oliver and Boyd, 1953, pp 393-402.

As regards gangrene and skin spot, comparatively little was known about their epidemiology in the 1950s but effective fungicidal treatments were available and used commercially, especially dipping tubers in solutions

of organomercury compounds. Details of this method are contained in papers by Graham (1960), Boyd (1960), and Boyd and Penna (1967). The difficulties with the technique lay in the need to wash tubers to remove soil (soil rapidly inactivates organomercurials), the toxicity of the mercurials, the problem of safe disposal of spent dipping solution, and the need to dry tubers. To avoid this method, an attempt was made at Agricultural Scientific Services to find a safe gas which could be introduced into bulks of tubers and which would be active against Phoma and Polyscytalum at least. The work resulted in the discovery of 2-aminobutane fumigation of potatoes (Graham and Hamilton, 1970) and its introduction into commerce in 1972.

This presentation does not deal in detail with all the various aspects of 25 years research and development work on blackleg, soft rot, and fungal diseases, but rather provides a summary and linking narrative stating briefly the purposes of the work, methods, what was found, and what impact it has had on commercial practice, set out chronologically. Details of the investigations are contained in various papers which have been published over the years, and which are included as Appendix 1. Moreover, the presentation does not aim to review the various diseases or the nature of the organisms involved. Regarding the bacterial pathogens, Erwinia spp., readers are referred to reviews by Starr and Chatterjee (1972), Chatterjee and Starr (1980) (mainly genetics), and Perombelon and Kelman (1980) (ecology of the soft rot Erwinias). An excellent review of potato tuber diseases in Britain is given by Boyd (1972). An equally good general summary of various aspects of soft rot in potatoes is given by Lund (1979).

PART 1

SECTION 1: BLACKLEG AND SOFT ROT: THE PERIOD 1955-1965

Introduction

Over this decade, much effort was devoted to a detailed basic study of disease etiology, pathology, and epidemiology; and also the bacteriology and identity of the causal organisms.

1.1 Bacteriology

The soft rot *Erwinia* bacteria had been fairly well characterised by 1955, mostly by American workers, and details were published in the 7th Edition of Bergey's Manual of Determinative Bacteriology (1957). However, it was not always possible to clearly distinguish between *E. carotovora* and *E. atroseptica*, though some tests appeared to work reasonably consistently. These were investigated by Graham and Dowson (1960b) who found, *inter alia*, that some strains producing blackleg disease only at higher temperatures (see para 1.3) could be distinguished by slow acid production from lactose and production of indole from trytophan (these organisms are now firmly classified as a separate species of *Erwinia*, namely *E. chrysanthemi*, which appears in the 8th edition of Bergey's Manual of Determinative Bacteriology, 1974). Later, Graham (1964a) published the results of a series of bacteriological tests on 57 isolates of soft rot *Erwinia* bacteria, concluding, *inter alia*, that *E. atroseptica* could usually be distinguished from *E. carotovora*, and also from *E. chrysanthemi*, an organism which was never found associated with potatoes in the United Kingdom. The taxonomy of other *Erwinia* spp. was discussed in the 1964 paper, and it was tentatively concluded that the conglomerate genus

Erwinia should be divided into two, leaving the genus Erwinia (sensu stricto) for those organisms causing galls, wilts and certain other diseases, whereas the soft rot bacteria would be placed in the genus Pectobacterium, as originally suggested by Waldee (1945). This view has never gained general acceptance (Graham, 1972), although some bacteriologists still maintain that Pectobacterium should be recognised as a separate genus (Brenner, Fanning and Steigerwalt, 1977).

1.2 Serology

Antisera prepared in a conventional way were used to detect soft rot Erwinias in potato tissue (Graham 1963a), for quick routine diagnosis. It was not possible to distinguish between E. carotovora and E. atroseptica with certainty by this method. More recently, the serology of these organisms in relation to potatoes has been studied in much more detail by Lazar (1972), Vrugginck and Maas Geesteranus (1975) and De Boer, Copeman and Vrugginck (1979).

1.3 Pathology

The pathology of E. atroseptica and E. carotovora had been investigated over many years by various workers in different countries, but there was no agreement as to whether E. atroseptica alone or whether both organisms could cause blackleg, whereas the pathogenicity of E. chrysanthemi to potato stems was unknown.

However, Graham and Dowson (1960a) found that there was a pathogenicity/temperature relationship, E. atroseptica causing blackleg at comparatively low temperatures (below 19°C), while E. carotovora and E. chrysanthemi caused the disease at higher temperatures (24.5°C or over). Although over the years, this relationship has been questioned (eg Erinle, 1975), it has generally been found to hold true in temperate climates, and it is

noteworthy that recent studies of organisms associated with potato blackleg in warmer climates show that E. carotovora and E. chrysanthemi are the major pathogens (Perombelon and Kelman, 1980). (Interestingly, Fig. 1 in the paper by Graham and Dowson (1960a) shows development of blackleg by E. carotovora strain 377 at high temperatures. The organism was later identified in this laboratory as the maize pathovar of Erwinia chrysanthemi (Brenner, Fanning and Steigerwalt, 1977)).

1.4 Factors affecting the expression of infection

In general, it was found that tubers affected by other diseases (including dry rot and gangrene) could give rise to more blackleg-affected plants than 'healthy' seed (Graham and Harper, 1967).

A major predisposing factor was shown to be the rate of application of fertilizer, more specifically that the amount of nitrogen (but not potassium or phosphorus) was critical; in general, the more N that was applied, the less blackleg developed on stems (Graham and Harper, 1966), whereas the converse was true for tubers - the higher the rate of application of N, the more soft rot developed (Harper, Boyd and Graham, 1963). The reasons for these effects are still not understood.

Tuber size affected the expression of blackleg infection, in that the smaller the tubers the less likely they were to give rise to blackleg disease (Harper, Boyd and Graham, 1963). Again the reason for this is not clear.

1.5 Ecology of the bacteria

In connection with any study of the occurrence of particular kinds of bacteria in the environment, a selective medium is essential. Salicin-bile salt medium was developed by Noble and Graham (1956)

for this purpose.

Using the medium, no soft rot bacteria could be isolated from soils in spring before planting nor could they be obtained from artificially infected non-sterile soils after inoculation in autumn, and testing in spring (Graham, 1958a).

Another selective medium, a MacConkey-pectate double layer medium was introduced by Stewart (1962); it was found to be much superior. This medium is widely used up to the present, although other selective media, which are said to be better than MacConkey-pectate medium (for example see Cuppels and Kelman, 1974), have been introduced. Using salicin bile salt medium and later the MacConkey-pectate medium, a detailed study of the occurrence of soft rot Erwinia in soils and surface waters was made during 1960-62. The results were never published, as they were uniformly negative, but reference to these findings is made in the papers by Graham and Harper (1967) and Graham and Hardie (1971). Some two thousand soil samples from numerous areas of Scotland were tested; all samples were taken by a soil corer down to a depth of about 12.0 cm.

By contrast, soft rot Erwinia were commonly found associated with potato tubers, whether obviously rotted, or apparently healthy. The method employed to detect the bacteria in sound tubers was to use them as their own enrichment medium by asphyxiating them. This was done by placing individual tubers in Kilner jars, filling the jars with water to the brim, and screwing down the lid to form an air-tight seal. Tubers were incubated for up to 14 days at room temperature, when samples of rotted tissue were removed, a large loopful suspended in a small amount of sterile water and loopful of the suspension then plated

on MacConkey-pectate gel. Details of these experiments were never published, but general reference was made to results in the papers by Graham (1962), Graham and Harper (1967) and Graham and Hardie (1971). Perombelon (1972) has given details of a larger number of experiments confirming and greatly amplifying these observations.

The absence of any symptoms in many tubers carrying infection has led to the description of blackleg and tuber soft rot as 'latent' diseases. This term is especially popular with farmers, and refers to the fact that the seller cannot see disease, which then can develop when in the hands of the buyer. The so-called 'latent' aspect of the disease simply refers to the fact that the incubation period is prolonged, often spanning the whole of the storage period - indeed the infection may never express itself as manifest disease (Graham and Harper, 1967). The fungal diseases, gangrene and skin spot and dry rot show the same kind of phenomena, and thus farmers have tended to lump these bacterial and fungal diseases together as 'latent' disease.

Using the selective MacConkey-pectate medium, it was shown that when the crop was in the ground, and especially towards the end of the growing season, it was possible to isolate both E. carotovora and E. atroseptica from soil in the vicinity of infected plants, and this led Graham and Harper (1967) to suggest that the major sources of contamination of daughter tubers were the rotting stems and mother tubers. However, Perombelon (1972) showed that the main source of bacteria was the mother tubers irrespective of whether or not stems were affected. It is, of course, well known that the great majority of mother tubers rot naturally, and it is also known that the rots are usually associated with soft rot Erwinias

(see Perombelon and Kelman, 1980). Many of the plants growing from rotten tubers never develop stem infection for reasons which are still not understood, although studies by myself showed that gum-like substances were often deposited in the xylem vessels at the base of the uninfected stems, which apparently formed a barrier to the ingress of bacteria.

1.6 Blackleg in crops grown from Scottish seed in warmer climates; chemical treatments

Scotland has for many years exported seed tubers of certain potato varieties to warmer countries including South Africa and Israel. (The South African trade has now almost ceased.) A general experience has been that, not infrequently, stocks have given rise to severe outbreaks of blackleg, in some cases as much as 80% plant infection in the field. The reason for this is not clear, but it may reflect the particular growing conditions in warmer climates, including use of irrigation. These circumstances are not fully described in the literature, but mention of them is made by Graham and Volcani (1961) and Graham (1962).

Because of these outbreaks, an attempt was made to achieve control by chemical treatment of tubers with organomercurials (methoxyethyl mercury chloride (MEMC), and ethoxyethyl mercury chloride (EEMC)), and also in mixture with the antibiotic, streptomycin, used as dipping solutions. The tubers treated in Scotland were then shipped to Israel, planted, and the blackleg content was compared with untreated material. However, no significant improvement was found, and it was concluded that bacteria were probably too deep-seated in tubers for them to be destroyed by the treatments (Graham and Volcani, 1961). In additional experiments carried out in Scotland during 1962-63, similar results were obtained, although in both cases, an attempt was made to bring about deeper

penetration of the chemicals by the addition of DMSO (dimethyl sulphoxide) to the dipping solution. These observations were never published because all the results were negative.

SECTION 2: BLACKLEG AND SOFT ROT: THE PERIOD 1966-1974

Introduction

The observation that, in common with other tuber diseases, blackleg and soft rot were apparently primarily tuber-borne in Scotland, suggested a possible means of ridding potato stocks from *Erwinia* by propagation from stem cuttings. The principle involved was that since generally speaking few (if any) bacteria entered stems until late in the growing period, especially when potatoes were grown early in the year under glass, it should be possible to produce stocks free from infection by growing plants quickly, and taking side shoot stem cuttings for propagation, thus leaving infection behind in the mother tuber.

This method was first practised by Rothamsted Station workers to produce stocks free from skin spot (Hide, Hirst and Griffith, 1969) and was adapted so that stem cuttings were tested for the presence of *Erwinia* before propagation. The process is described in the paper by Graham and Hardie (1971); an outline is given below (para 2.2).

Studies also continued on the bacteriological characters of what was by now a large collection of *Erwinia* spp.

2.1 Bacteriology

During the period 1966-1974, a large representative collection of isolates of soft rot *Erwinia* bacteria was built up and subjected to a series of physiological and biochemical tests in the hope of finding additional tests which would reliably distinguish between *E. carotovora*,

E. atroseptica and E. chrysanthemi. The results are detailed in the paper on identification of soft rot coliform bacteria by Graham (1972), which was to some extent based on the earlier work of Dye (1969).

One of the most important tests for distinguishing E. carotovora and E. atroseptica proved to be the formation of reducing substances from sucrose; E. atroseptica produced them, while E. carotovora did not. This reaction was discovered empirically by Moustafa and Whittenbury (1969), who subjected some strains of soft rot Erwinia bacteria to tests designed to distinguish between species of Pseudomonas. Many more strains were examined in this laboratory and the test was found to be remarkably consistent, although aberrant strains can occur (Graham, 1972). Moustafa and Whittenbury (1969) found only one reducing substance using paper chromatographic analysis, but two were found when culture filtrates were analysed by thin layer chromatography in the Chemistry Department at this laboratory (referred to by Lund and Wyatt, 1973). One spot gave a yellow colouration, the other a reddish colouration with aniline-diphenylamine reagent. Neither were 3-ketosucrose. The identity of the reducing compounds was determined by Lund and Wyatt (1973); the substance giving the red colouration was 1-α-glucosylfructose, while the yellow colouration was due to the presence of 6-α-glucosylfructose (palatinose). Some other Erwinia species were found to form reducing substances from sucrose, but these proved to be mainly mixtures of glucose and fructose (Lund, 1975).

Most of the tests described by Dye (1969) and Graham (1972) are incorporated into the scheme for identifying Erwinia spp. given in Bergey's Manual of Determinative Bacteriology, eighth edition, 1974.

2.2 Outline of the stem cutting procedure

Because of the great concern about the health standard of Scottish seed potatoes it was decided administratively in 1966 that a large scale attempt should be made to produce pathogen-free stocks, without awaiting the results of detailed further research, which would have been devoted to testing and evaluating the method, and which could have taken some years. Moreover, it was thought that other ways by which blackleg and soft rot bacteria might spread in the environment would only be uncovered when large numbers of disease-free plants were exposed in the field.

Accordingly in 1967 DAFS rented a farm (known as Ingraston) some 25 miles south-west of Edinburgh, in an upland area where almost no potatoes were grown. The process of stem-cutting production was as follows. Plants were raised in the spring from tubers planted in February/March in glasshouses. When plants were about 30 cm high, the growing point was removed, causing the axillary buds to develop. When the side-shoots were about 5-8 cm long, they were removed and kept in damp paper towelling. The stem base (about 1 cm) was removed from the cutting taken at the lowest point on the stem, and tested for the presence of Erwinia by crushing the stem and plating out the sap on MacConkey-pectate medium. The plates were incubated for up to 48 h at 26°C to allow colonies to develop. If the plates were negative, the cuttings (which had been held in wet paper towelling) were then rooted under mist propagation, transplanted into 7 cm pots and kept in the glasshouse until well established, then hardened off in frames, before being hand

planted in the field as separate clones (ie the cuttings taken from each plant were treated as a separate clone). This procedure may be called the "classical" method, since various new methods have been introduced more recently, but nevertheless, the principle remains essentially the same.

The effect of this procedure was immediately plain, for in 1967 none of 1,000 cuttings planted in the field were affected by blackleg. Indeed the process was so successful that it was decided to produce as much as possible of this pathogen-free nuclear material, and with the agreement of the appropriate bodies representing farmers and merchants, a new grade of elite material, referred to as "Virus Tested Stem Cuttings" or VTSC grade, was introduced into the Potato Classification Scheme. This nuclear VTSC material was first released to the growers in 1970. By the year 1980, almost all stocks of seed potatoes in Scotland were derived from the nuclear VTSC material.

Details of the VTSC scheme and its requirements are contained in official DAFS publications for growers of certified seed. These are revised annually. In addition, two booklets were issued for growers by DAFS in 1970 entitled "Potato Growers Guide to Commercial Seed Production" and "Potato Growers Guide to Clonal Selection", both written by Dr J L Hardie, in his capacity as Head of the Potato Section at Agricultural Scientific Services.

2.3 Summary of results showing effectiveness of the stem cutting method in controlling blackleg and soft rot at the DAFS nuclear stock farm

Since the production of VTSC nuclear stock began, a careful study has

been made into any infection with Erwinia which occurred at Ingraston Farm. Details of the results over the years 1967-74 were published by Graham, Quinn, Sells and Harrison (1976). In essence, these showed that infections did occur with both E. carotovora and E. atroseptica, though they were rare. Moreover, most visible infections were soft rots on stems above ground level, and yielded mostly E. carotovora. This was especially notable when stem rot was found in first year stem cuttings, as in 1971 (Graham and Hardie, 1971 - but note that at that time the organisms were wrongly identified as E. atroseptica).

2.4 Association of Erwinia bacteria with insects

2.4a On the nuclear stock farm

The foregoing observations showing that above ground plant parts were found naturally infected, suggested that some kind of airborne spread was taking place, and it was thought that insects might have been responsible, particularly as there were scattered reports over the years in phytopathological and entomological literature suggesting that insects were contaminated with soft rot Erwinia, and that Erwinia infection could be spread by them (for relevant references see Graham, Quinn, Sells and Harrison, 1976).

In 1972 a search began for insects carrying soft rot Erwinia in the Ingraston environment and insects were caught in a continuously operating suction trap set in the VTSC potato

field. However, this method proved unsatisfactory, because the insects dried too quickly, and bacterial populations fell sharply as a result. In the years 1973 and 1974 live insects were caught in and around Ingraston Farm and tested according to the method described by Graham, Quinn, Sells and Harrison (1976).

At a vegetable waste dump outside the confines of Ingraston, insects (mainly species of Leptocera) were found contaminated in July 1973, and they continued to be caught until the middle of October of that year, by which time insect activity was greatly reduced. The organisms found were identified as E. carotovora only. In August 1973, stem rots caused by E. carotovora were found in the stem cutting material, and using an arbitrary serogrouping procedure, it was shown that the same serogroups were found associated with insects and the stem infection (Graham, Quinn, Sells and Harrison, 1976). This, coupled with experimental evidence that insects contaminated with Erwinia could transmit them to damaged stems (Harrison, Quinn, Sells and Graham, 1977) provided compelling though not conclusive evidence that the infections on potato stems arose from transmission of the organisms by the insects. It was also notable that not all serogroups found in association with stems could be found on insects, but this might merely have been due to the fact that comparatively few insects were tested and thus the other serogroups of bacteria were undetected, or alternatively that organisms were reaching the stems through the air in some other way (Graham, Quinn, Sells and Harrison, 1976). Additionally, and

perhaps most importantly, E. atroseptica was never found associated with insects, although this organism was also found at Ingraston in infected stems (Graham, Quinn, Sells and Harrison, 1976). This was also considered to be further evidence that organisms infected plants in some other way, possibly through the atmosphere.

2.4b Contamination of insects associated with waste potato dumps

Many waste potato dumps occur in the general environment of seed potato growing areas, and it was thought that they might be important sources of contaminated insects dispersing into the environment. Accordingly, observations on insects at two large dumps during 1973 and 1974 were made. These showed that up to 5.7% of dipterous insects caught at the dumps were contaminated with E. atroseptica or E. carotovora (or occasionally, both), the majority of the organisms being E. carotovora, despite the fact that most of the organisms infecting the tubers when originally dumped would most probably have been E. atroseptica (Harrison, Quinn, Sells and Graham, 1977). Altogether insects in 12 genera in the Order Diptera, representing at least 13 species were found contaminated.

Waste dumps are also known to be primary foci of infection with viruses and potato blight disease (Phytophthora infestans) (Boyd, 1974), and these observations on bacteria re-emphasised the need for growers to avoid making or otherwise destroying

waste potato dumps.

2.5 Spread of Erwinia by farm implements

In common with many other potato diseases, Erwinia infections could be spread by contaminated farm machinery. Results showing two aspects of husbandry which can cause infection is given in Graham and Hardie (1971); these are use of contaminated graders, and by contaminated tractors which pass through the crop when spraying for potato pest or disease control (fig. 1).

SECTION 3: BLACKLEG AND SOFT ROT: THE PERIOD 1975-1979

Introduction

During this period, most work was devoted to studying the ecology of Erwinia in relation to contamination of pathogen-free stocks. As pointed out in Section 2.4, re-infection of nuclear stocks at Ingraston with E. atroseptica and possibly some serogroups of E. carotovora could not be explained solely on the basis of insect transmission, and the impression was gained that the organisms might be present in the atmosphere. Conceptually the difficulty lay in appreciating how Erwinia could become aerosolized by natural physical or biological processes. Plainly, spread within crops would take place by rain splash, but as far as was known, the droplets would be comparatively large and have a ballistic trajectory so that they would fall only within the crop and immediately around the crop's edge. This had already been found to be the case by myself, for in 1962 MacConkey-pectate plates placed upside down on holders at the edge of a potato

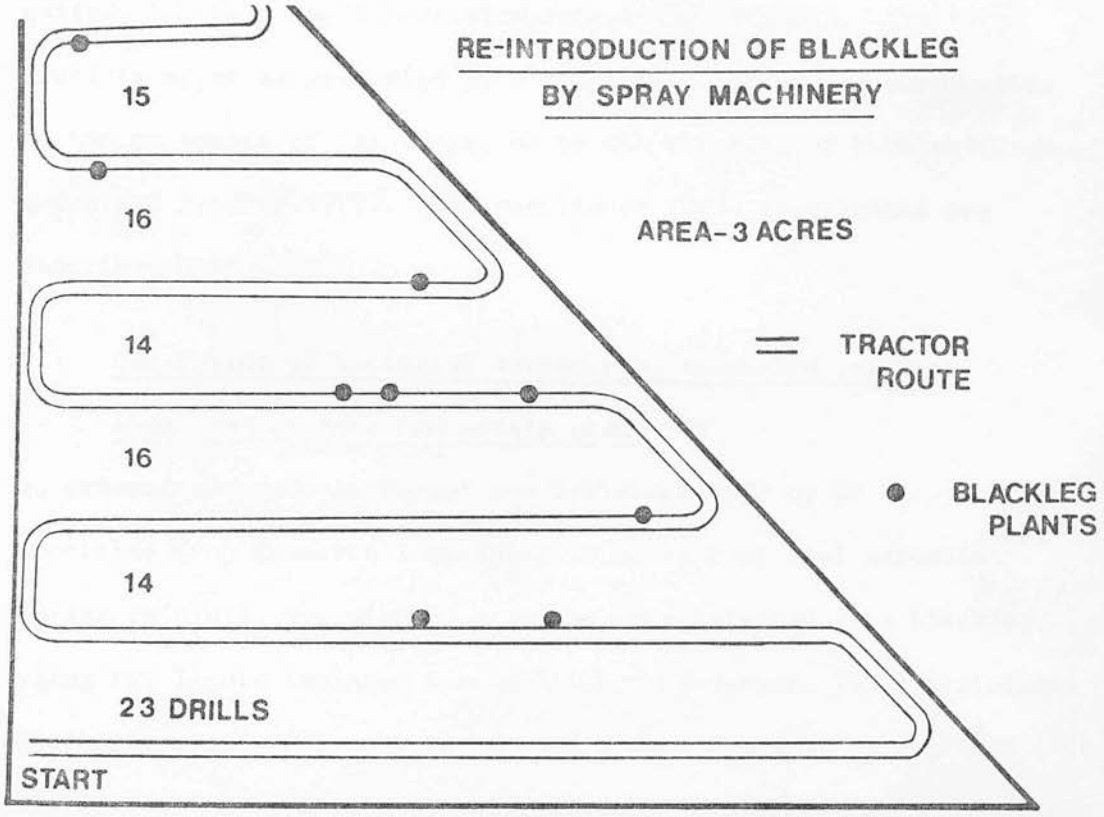

Fig. 1. Blackleg-infected potato plants along tractor wheel tracks.

crop, were contaminated by soft rot Erwinia during rainfall (these results were never published because this was merely a fragmentary observation and which was not unexpected).

However, it was thought possible that some tiny droplets might be formed during impact of raindrops, which would evaporate very quickly and form small particles containing bacteria. The tiny droplets might be generated by a coronet-shaped upward surge which occurs on impact of raindrops, or by the bursting of bubbles (Graham, Quinn and Bradley 1977)*. The results of these experiments are described below.

3.1 Generation of bacterial aerosols by simulated raindrop impaction on infected potato stems

An attempt was made in August and September 1972 by Dr Perombelon (Scottish Crop Research Institute) to catch bacterial aerosols during rainfall, downwind of a potato crop infected with blackleg, using MRE liquid impinger samplers (May and Harper, 1957) containing MacConkey broth. No soft rot Erwinias were found by this method, but plates were covered with colonies of other bacteria. This was probably due to the fact that August and early September 1972 was a dry period and dust was often projected into the air at the beginning of rainfall or blown by wind. This dust was probably the source of many of the organisms.

As the method proved ineffective, it was thought possible that very few Erwinia bacteria might be projected into the air, and that they would be easily missed amongst the many other bacteria caught in the

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* But see Addendum

liquid impinger. It was therefore decided to build a special piece of apparatus to see if an aerosol could be generated in a closed system by impaction of water drops on infected potato stems. It was also designed to test for generation of aerosols small enough to be carried in slow air streams along a wind tunnel, and which could subsequently be caught using Casella Airborne Bacteria Samplers, sampling at different points along the length of the tunnel.

This apparatus is described and figured in the paper by Graham and Harrison (1975). It consisted of a stainless steel tank placed at the bottom of a high spiral staircase. A plastic tube, down which water drops could fall, extended to the top of the staircase (about 7.5 m). At this height drops 2 to 5 mm in diameter would either reach or almost reach terminal velocity. Humidified air was fed into the tank, and flowed out along a wind tunnel 4.1 m long. Air was drawn through the system by a fan fixed at the end of the wind tunnel. Sampling tubes, which could be connected to Casella samplers, sampling air at 30 l/min, were placed at intervals along the tube.

Water drops varying in size from 2 to 5 mm in diameter were allowed to fall on to potato stems infected with E. atroseptica.

The results showed that aerosols were produced by all drop sizes, but larger drops, having higher kinetic energy, generated most airborne particles. It was also found that viable particles could remain suspended in the air in the system for 1 h or more, showing that they were small, probably in the range of 4 to 8 µm in diameter. In all probability, the particles would be free bacterial cells since in

very small droplets the water evaporates very rapidly, even at 100% r.h., because vapour pressure is raised at a curved surface (the so-called Kelvin effect). The number of bacterial cells in each particle would vary depending on the size of the droplet and probably other physical parameters. These particles are referred to as propagules (in the USA, the term colony-forming units (cfu's) is preferred).

3.2 Quantitative determination of the number of viable propagules generated per unit weight of infected stem

The apparatus described by Graham and Harrison (1975) could not determine the number of viable propagules in unit volume of air as sampling was anisokinetic, that is to say the velocity of air in the sampling tube differed from the velocity in the wind tunnel (May 1967). All sampling tubes were removed, and the apparatus modified to achieve isokinetic sampling by inserting a specially designed sampling tube into the tunnel, as described by Graham, Quinn and Bradley (1977). Using this modified apparatus, the number of propagules in unit volume of air generated per unit weight of infected stem was easily determined - details are given in Graham, Quinn and Bradley (1977). Briefly, results showed that as many as 1,039 propagules were generated per gram of infected stem using 5 mm diam drops.

The particle size distribution was determined with an Andersen sampler (Andersen, 1958). The results demonstrated that most propagules fell into the range 1.1-4.7 μm, whether 3 mm or 5 mm drops were used. This implies that many viable propagules were free bacterial cells, as originally suggested by Graham and Harrison (1975).

3.3 Survival of bacteria in small particles in the laboratory and in the open air

It was known that, in a closed system, some bacteria would survive for 1 h or more, when suspended as small particles (Graham and Harrison, 1975). However work by scientists studying survival of airborne bacteria at the Microbiological Research Establishment, Porton Down, had already shown that survival of various organisms in the open air need not be related to that in a closed system (for instance, see Druett, 1973). In general, bacteria did not survive so long in the open air, which was at least partly due to the presence of toxic material in the atmosphere - generally referred to as the 'Open Air Factor' or 'OAF'. The precise nature of OAF is unknown, but is mostly to be found near towns, and it appears that some, if not all, the OAF is made up of olefine ozonides, which are highly chemically reactive (Druett, 1973).

The method used to determine survival in the open air was the so-called "captive aerosol" technique, originally described by May and Druett (1968). The bacteria are loaded on to microthreads of spider gossamer wound on stainless steel frames, which can then be exposed to the open air, and the decline in viability determined.

Graham, Quinn, Sells and Harrison (1979) applied the method to strains of E. carotovora and E. atroseptica isolated from different environments in Scotland and Colorado, first in a controlled environment room and then in the open air. The results showed that although the bacteria lost viability more quickly than a robust reference strain

of Escherichia coli, sufficient numbers survived for 15 min. or more to indicate that airborne spread of viable propagules over a distance could occur, especially under the cool humid atmospheric conditions which commonly occur in Scotland. The bacteria from Scotland and Colorado behaved in much the same way, and the isolates of E. carotovora showed evidence that they were rather more robust than those of E. atroseptica.

3.4 The search for Erwinia bacteria in the atmosphere

Because experiments with the raindrop simulator/wind tunnel showed the formation of many viable propagules from relatively small amounts of rotted tissue, and the "captive aerosol" investigations showed that bacteria could survive in a viable state for 15 min. or more, it seemed likely that they could be caught from the air down wind of potato crops during periods of rainfall, when infected stems were present. Experiments along these lines were carried out over two years using a Casella High Volume Airborne Bacteria Sampler (sampling at 700 l/min), the air being sampled at various times of year at various sites, when it was raining or dry (Quinn, Sells and Graham, 1980.

The results demonstrated that viable cells of E. carotovora and E. atroseptica were commonly present in the air near Edinburgh and in East Lothian from late summer to early winter, but not in late winter, spring or early summer. At times large numbers of bacteria were caught at sites where no potato crops could be seen nearby; and it was also noteworthy that the bacteria were found in late autumn

and early winter long after potatoes were lifted and haulm dead. This suggested there were sources of the bacteria other than potatoes. These sources have not so far been discovered, but one could be overwintering brassicas, plants which are susceptible to attack by Erwinia.

About the same time, Perombelon, Fox and Lowe (1979) found that haulm pulverisation caused considerable production of aerosols, but as distinct from rainfall, this is a once and for all process. Furthermore, haulm debris left after pulverisation prior to harvest could be a major source of tuber contamination in that crop.

3.5 Consideration of particle deposition gradients from quantitative data for interpretation of gradients in terms of separation distances between crops to avoid contamination by bacterial aerosols

Using the mathematical model of Gregory (1961) to calculate deposition gradients, Graham, Quinn and Bradley (1977) derived numbers of bacteria deposited on unit area at several arbitrary distances downwind up to 1 Km, assuming a certain area of crop (one hectare) and where 2% and 10% of plants were affected by blackleg disease. Ignoring the fact that the bacteria will lose viability in the atmosphere, the results suggested that for both point and area sources, considerable numbers of bacteria could be deposited at distances up to 1 Km. There are, however, other mathematical models which can be used, notably that of Pasquill (1974). Using this model, which gives the numbers of particles in unit volume and from which the

the number of bacteria deposited per unit area can be found using the mathematical procedures described by Chamberlain (1966), calculations show that far fewer bacteria are deposited per unit area. Perombelon, Fox and Lowe (1979) calculated that, using the same data as for the Gregory model, only about 5 propagules would be deposited per m^2 at 1Km from the source under stable atmospheric conditions. Nevertheless, taking into account the practical field situations described by Quinn, Sells and Graham (1980), airborne spread is likely to be an important way that Erwinia-free nuclear stocks can be contaminated.

In practice these physical phenomena must be gigantic processes, where large areas of crops are struck by large numbers of raindrops, causing a great many bacteria to be projected into the air. At times, especially during heavy rainfall in late summer and autumn, there appear to be clouds of aerosol blowing on the wind. This is illustrated by the large number of soft rot Erwinia colonies found in some of the experiments described by Quinn, Sells and Graham (1980).

Another factor is the "scrubbing" effect of rain. The physical processes are described by Chamberlain (1967); results show that rain, although a good aerosol generator, is rather inefficient at removing small airborne particles. Even so, rain must carry down some of the propagules.

The importance of airborne spread when considering effective separation distances to minimise contamination of VTSC stocks should

not be underestimated. At present, separation distances defined in the Classification Scheme are set at only a few metres, mainly to avoid direct contact between the potato clones, and to enable easy cultivation by machinery to minimise damage. The kind of distances needed to minimise contamination, say up to 500 m at least, are not commonly achievable in seed potato growing areas and it is hard to see how spread could be dealt with at the present time (this is discussed further at paragraph 5.3).

It is known that artificially generated aerosols of a certain arbitrary serogroup of E. carotovora released up wind, deposited on leaves of potatoes in the open air, and that Erwinia of the same serogroup were found associated with the progeny tubers (Graham, Quinn, Sells and Harrison, 1979). Perombelon (1978) has shown that following deposition of Erwinia propagules on potato leaves, bacterial cells can multiply there in wet weather, and then be washed down into soil to contaminate progeny tubers.

3.6 General consideration of the generation of atmospheric bacterial aerosols

Since rainfall can cause the projection of Erwinia bacteria into the air to form aerosols, other organisms found on plant surfaces or in soil must be projected into the air in the same way. This was observable in the Erwinia experiments, even when using the MacConkey-pectate medium, because many bacteria which were not pectolytic appeared on the plates, their numbers increasing or decreasing with the heaviness of the rainfall, then falling sharply when rain ceased.

To study this phenomenon more closely, an experiment was done at one site by taking samples at 15 min intervals before, during, and after rainfall ceased, over a 24 h period, using MacConkey-pectate medium. These data have not been published, although some general comments concerning this aspect were made in the paper by Quinn, Sells and Graham (1980), namely that by analogy rainfall must be a major generator of the general atmospheric aerosol. The methods and results referring to the experiment are described below.

Air samples were taken at 15 min intervals for 24 h, using a high volume Casella Sampler sampling at the rate of 700 1/min. Meteorological data were also recorded, the rainfall being measured with an automatic rain gauge. The result is illustrated in fig. 2, where two 15 min counts are summed to make a 30 min count, for ease of presentation. The rise and fall of the total bacterial count related to rainfall is clearly seen. However, in this case, it was very notable that after rain ceased, and the total bacterial count had fallen to low levels, it then rose and continued to rise over a long period. The bacteria involved were found to be a single kind of organism, a species of Micrococcus, its numbers rising far above the total bacterial count during rainfall. The source of the Micrococci is unknown, but plainly there must be other ways than rainfall by which atmospheric bacterial aerosols can be generated. Some of these are mentioned by Akers, et al., (1979); moving vehicles (the rotation of wheels particularly on a wet surface must be an effective aerosol generator), various industrial processes,

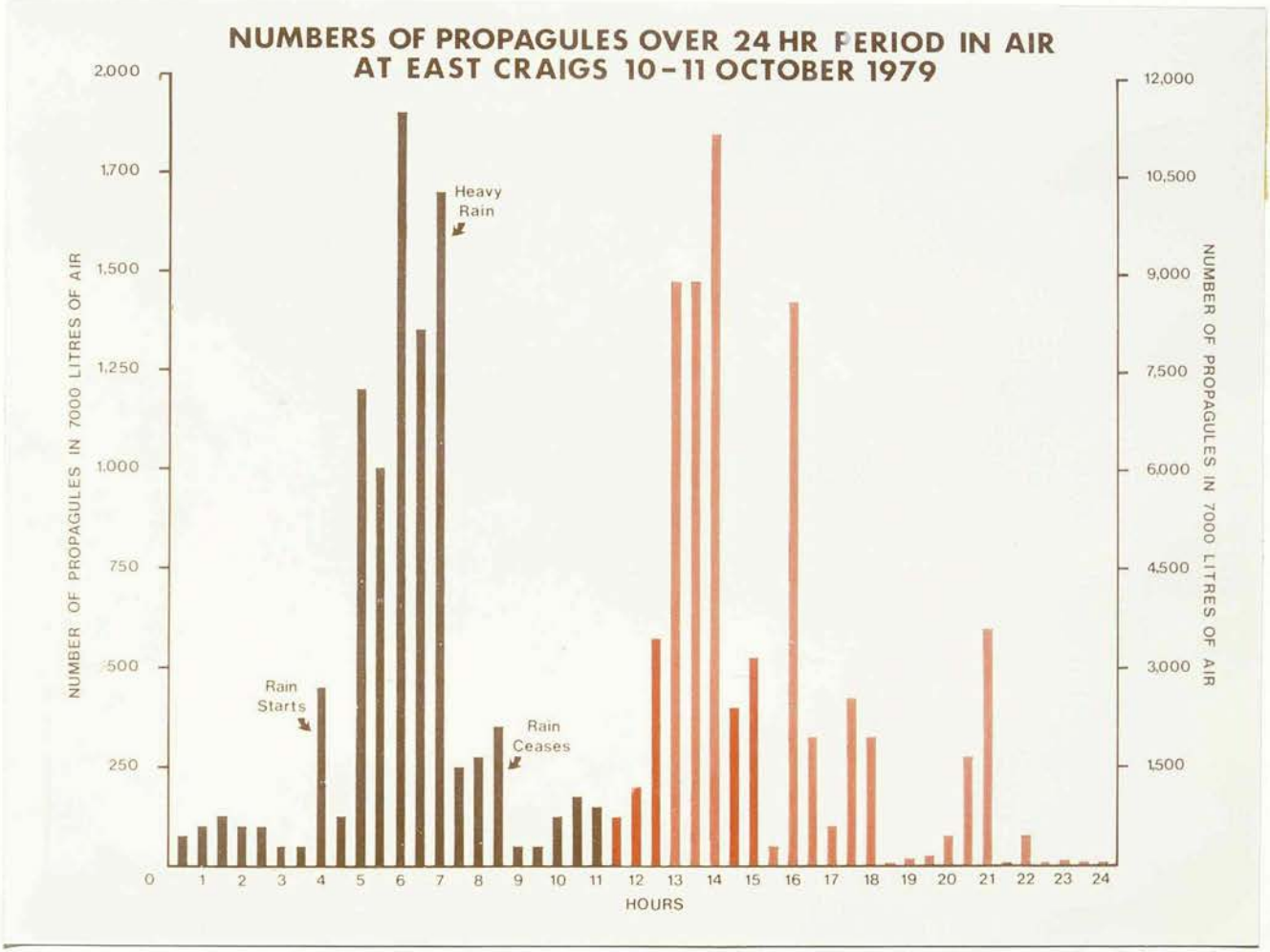

Fig. 2. Bar graph illustrating changes in the numbers of bacteria in the air over a 24 hr. period, associated with rainfall and the absence of rain. Black bars - total count of all bacteria (left hand scale). Red bars - total count of micrococci (right hand scale).

including the operation of cooling towers and sewage treatment plants.

SECTION 4: BLACKLEG AND SOFT ROT: THE PERIOD 1979-1980

Introduction

With the development of chemically defined liquid enrichment media incubated under anaerobic conditions, combined with the use of selective media, the time was ripe for a re-examination of soil (particularly in plant rhizospheres) and waters for the presence of Erwinia. This technique greatly increases the chances of detecting very low numbers of Erwinia cells.

4.1 Use of liquid enrichment media for detection of soft rot Erwinia

The method in this laboratory involves use of liquid enrichment methods under anaerobic conditions, coupled with use of the MacConkey-pectate selective medium. The enrichment media, such as those described by Meneley and Stangellhini (1976) and Burr and Scroth (1977), depend on the use of polygalacturonate as a sole carbon source, with inorganic salts, and a surface active agent such as Tergitol 7. All experiments in this laboratory employed the medium described by Burr and Schroth (1977); the tubes were incubated for 48 h at 25°C under anaerobic conditions ("Gaspak" equipment is the most suitable) before plating on to a selective medium.

To avoid aerobic growth in tubes after the water or root samples had been taken, the "Gaspak" anaerobic jars were taken to the field, and as

jars became full, they were closed after the hydrogen source was introduced.

4.2 Occurrence of Erwinia bacteria in the rhizosphere of weeds and crop plants (including potato)

In recent years, several workers in North America and in Japan and USSR have shown that E. carotovora and E. atroseptica can be isolated from the rhizosphere of weeds and crop plants (Meneley and Stanghellini, 1976; Burr and Schroth, 1977, Kikumoto and Sakamoto, 1969)(for a fuller account see Perombelon and Kelman, 1980). Because the environments in which these observations have been made are not strictly comparable with those found in Scotland, and because it was difficult to assess the significance of the observations in relation to the epidemiology of potato blackleg and soft rot, it was decided to carry out similar experiments in Scotland. Much of this work was undertaken by Mrs N J McCarter-Zorner, of Colorado State University, and I wish to record my indebtedness to her. (Mrs McCarter-Zorner had previously studied the situation in Colorado, where E. atroseptica and E. carotovora were also found in association with weed roots; these data have not yet been published).

In the 1980 experiments, pieces of roots of approximately 50 weeds and crop plants plus the adhering soil were sampled at two to three week intervals from early June until mid-October, 1980 at 16 different sites where potatoes had been grown in 1979; most of the sites had cereal crops growing there but one with a potato, one with a raspberry crop and two with turnips were included. The results are summarised in the bar graph (fig. 3).

They show that in most cases, Erwinia spp. were not found until the end of July, and then in the rhizosphere of only a few plants in a few

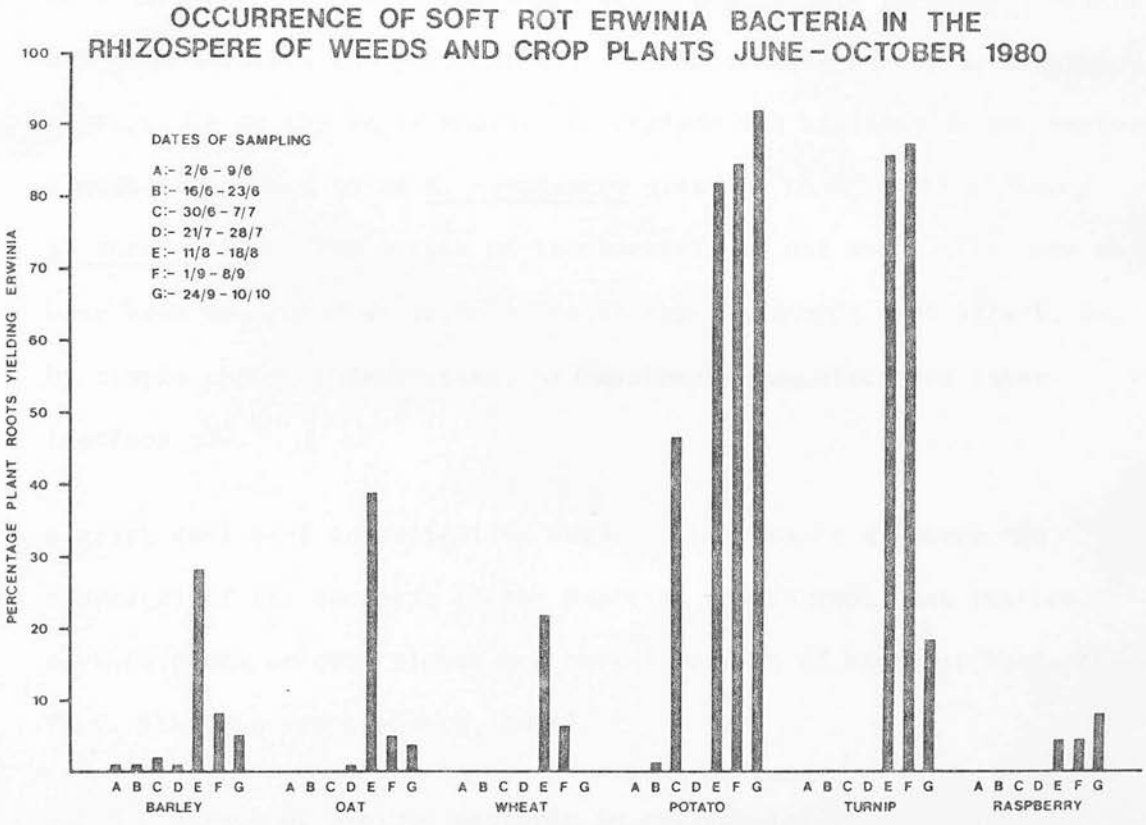

Fig. 3. Bar graph showing rise and fall of populations of Erwinia bacteria in the rhizosphere of crop plants and weeds during the season June–October 1980.

fields. However, generally by mid August the number of weeds and crop plants yielding the organisms rose dramatically, which was followed by an equally dramatic fall in September and October. In the case of the single potato crop and the two turnip crops, high numbers of root samples yielding Erwinia continued into September and October. The reason for this is not clear, but it may be due to the fact that potatoes and turnips are crops susceptible to Erwinia attack and thus maintain the populations, whereas cereals and weeds are not susceptible so far as is known. As regards the identity of the bacteria, almost all proved to be E. carotovora with $>$ 1% of tests yielding E. atroseptica. The origin of the bacteria is not known, but some may have been brought down in rain due to the "scrubbing out" effect, or by simple physical deposition, or from other sources discussed later (Section 5).

A great deal more investigation needs to be done to discover the source(s) of the bacteria in the Scottish environment, and whether certain weeds or crop plants are better sources of bacteria than others (c.f. Kikumoto and Sakamoto, 1969).

4.3 Presence of Erwinia bacteria in surface waters

In connection with the studies of bacteria in association with weed roots, aquatic plant roots were sampled beginning in June in ditches around the edges of fields. Surprisingly, a very high percentage (sometimes around 90%) of plants yielded the bacteria. Subsequently it was found that water in ditches was contaminated with Erwinia. In this case bacteria were detected by adding equal volumes of water (c. 50 ml) to double-strength polygalacturonate enrichment medium, and incubating for 48 h anaerobically. Details are described in an

as yet unpublished paper by McCarter-Zorner, Quinn, Sells and Graham, reproduced at Appendix 2. Briefly, it was found that very many surface water samples from ditches, drains, streams and even large rivers were contaminated with Erwinia from June through November. Observations will continue throughout winter and spring to find out if the bacteria occur in surface waters throughout the season. It is notable that the organism found in almost all water samples was E. carotovora; E. atroseptica was found only occasionally.

Another very important finding was that the bacteria were often present in streams in upland and mountainous areas, where the only kind of agriculture was sheep farming. The source(s) of these bacteria are unknown, but, as in the case of Erwinia bacteria in association with weed and crop plant roots, some at least may have been deposited from the atmosphere. However the air is most unlikely to be the sole source because bacteria were readily found in only 50 ml of water taken from large rivers, where the dilution factor must be very great. The possible origin of the bacteria is discussed later (Section 5).

4.4 Infection of "bait" potato plants grown in different environments

During the past two years, Erwinia-free potato plants have been placed in different environments to see if or to what extent they became infected with Erwinia. Site 1 was in the midst of an arable area where numerous potato crops occurred nearby, Site 2 in an upland area where there were no potato crops for several kilometres, and Site 3 in a remote mountainous area of the Southern Uplands, with sheep farming.

Fifty plants grown from tested stem cuttings were exposed at each site during the whole of the growing season, and infection of tubers from each plant was determined by anaerobic rotting and isolating bacteria in the usual way.

Results showed that Erwinia had infected plants at all 3 sites in 1979 and 1980. The number of infected plants from each site in 1979 and in 1980 was as follows (1980 figures in parenthesis) site 1, 23, (21); site 2, 16, (48); site 3, 31, (4). The bacteria were identified, with the following results: site 1, 22, (21) isolates were E. carotovora, 1, (0) was E. atroseptica; site 2, 16, (48) isolates were E. carotovora, 0, (0) were E. atroseptica; site 3, 31, (4) isolates were E. carotovora, 0, (0) were E. atroseptica.

Some larger bait plots in virgin soil were grown at Agricultural Scientific Services in 1979 and 1980; this site is on the outskirts of Edinburgh, near to agricultural land. The results were as follows (1980 in parenthesis). Number of bait cuttings planted 116, (115); number infected 21, (87); number infected with E. carotovora 1, (86); number infected with E. atroseptica 20, (1).

These preliminary results indicate that there was great variation in infection levels, but the main conclusion so far is that even if the nuclear stocks were grown in remote areas (as some growers and scientists have suggested) there is no guarantee that the crop would be free from soft rot Erwinia infection. However it is noteworthy that infection at most sites and in most years was of E. carotovora only, but these results were not entirely unexpected, as viable aerosols could presumably spread over considerable distances, as could infected insects, and E. carotovora is more robust and survives better than E. atroseptica. Furthermore, E. carotovora (as noted at 4.3) was commonly isolated from surface waters in the upland and remote areas as well as in arable areas.

SECTION 5: DISCUSSION; FURTHER INVESTIGATIONS AIMED AT CONTROLLING BLACKLEG AND SOFT ROT

Introduction

A striking feature of the results of research over the last 25 years is that the ecology of the soft rot Erwinias and the epidemiology of the diseases they cause are very complex, and are still far from being fully understood. Moreover as pointed out by Perombelon and Kelman (1980), ecology and epidemiology will vary according to the nature of the climate and the pattern of agriculture. In this connection, it is now known E. chrysanthemi is a cause of blackleg in warmer climates; the first record of the association of this organism with blackleg in warmer areas was when Graham (1972) reported finding this organism amongst a collection of isolates from potatoes in Brazil, received from Prof. C F Robbs in 1964.*

5.1 Monitoring infection in commercial VTSC potato stocks

The discovery that E. carotovora is in the atmosphere; in association with weed and crop plant roots; and also in surface water, suggests that the bacterium is widespread in the environment and it is therefore likely that, in time, pathogen-free stocks would become infected. E. atroseptica is much less common in the environment, and thus, in general, potato stocks are not likely to become infected so rapidly as with E. carotovora. Monitoring of infection when VTSC stocks are in the hands of commercial growers is one of the important aspects needing continuing investigation. Much of the monitoring work is being done by Dr Perombelon of the Scottish Crop Research Institute, together with Mr Quinn and Miss Sells (Mrs Lindsay) at Agricultural Scientific Services, and a paper has been published

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* Footnote: A Tanii & T Baba (Bull. Hokkaido Pref. Agr. Exp. Stations, 24, 1-10, 1971) reported on a bacterial stem rot of potato in Japan caused by E. chrysanthemi, but from the description given, the identity of the organism is uncertain.

describing the results so far (Perombelon, Lowe, Quinn and Sells, 1980). This study, which has now extended over six years at various farms, shows that Erwinia-free nuclear VTSC stock produced by DAFS tended to become infected with Erwinia carotovora more than E. atroseptica, although the rapidity with which this occurred varied from farm to farm for reasons not clearly understood (that is to say, there were often no very obvious differences in the methods of standards of husbandry). As suggested by Perombelon, Lowe, Quinn and Sells, (1980), a close study of soil water status, agronomic methods, and the environment generally on "good" and "poor" farms respectively, could indicate reasons for the differences, which might be easily translated into improved practice.

Nevertheless, despite the danger of re-infection the fact remains that the introduction of VTSC has generally coincided with reduced amounts of blackleg in Scottish potato stocks. This is illustrated by the levels of blackleg found at official inspection (mid-July to mid-August) over the years 1964-1979 referred to in the paper by Perombelon, Lowe, Quinn and Sells, (1980). Another notable feature is that disease levels have become less erratic since substantial quantities of VTSC and VTSC-derived material appeared in commerce around 1973. The graph (fig. 4) shows the percentage of all stocks (VTSC+FS+AA grades) which reached the FS blackleg tolerance of 0.25% infection from 1964-1979. However, other factors may well have played a part in reducing blackleg, such as changes in the relative importance of different potato varieties and introduction of new varieties which vary in susceptibility, changes in cultural and storage practice, and weather conditions, especially the dry years of 1975 and 1976.

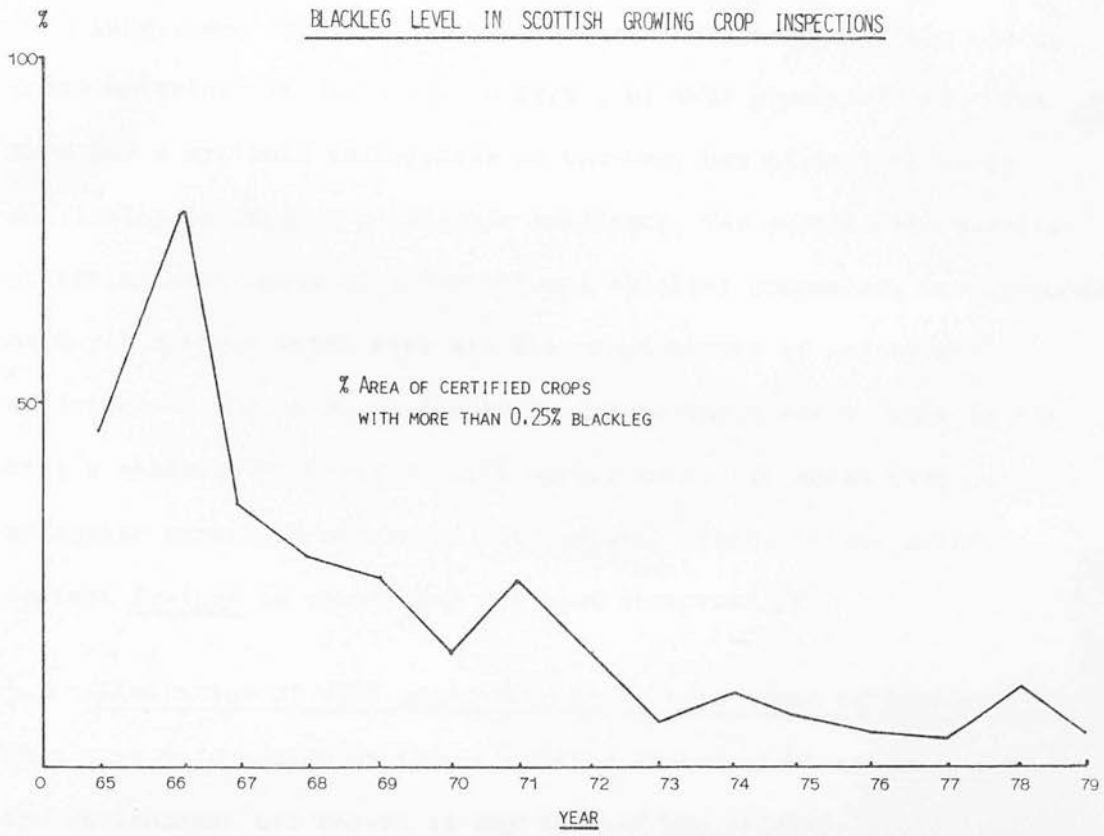

Fig. 4. Graph showing blackleg incidence in potato crops at inspection time over the years 1965 to 1979.

5.2 Chemical control of Erwinia infections

At the time of writing (early 1981), a major difficulty with blackleg and soft rot control lay in the fact that there are no known effective anti-bacterial chemical treatments available to integrate with the stem cutting procedure. This is in contrast to the situation regarding fungal diseases, where VTSC and other grades are regularly treated with fungicides such as 2-aminobutane and thiabendazole (the use of these materials is discussed in Part 2 of this presentation). The need for a systemic bactericide is obvious, but plainly there is difficulty in finding a suitable substance, for despite the massive screening programmes of international chemical companies, no compounds have yet emerged which have all the requirements of safety and efficiency. Moreover, so far as is known, there are no indications that a systemic bactericide will appear soon. It seems that no molecular structure necessary for systemic antibacterial action against *Erwinia* in plants has yet been discovered.*

5.3 Limitation of VTSC production to certain areas of Scotland

When more information on the occurrence and survival of *Erwinia* in the environment are known, it may be that the evidence would lend support to the concept of designating geographical areas where VTSC production should be concentrated. In such circumstances, it is hard to see growers and merchants accepting this in practical, financial

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*Footnote: The destruction of *Erwinia* cells within tuber lenticels using chloropicrin has been reported by Kloepper et al., (1979), but this substance is much too toxic for general use - see remarks on p.81.

and political terms. On the one hand it would be likely that the designated areas could not be used to grow any potatoes except VTSC, while, on the other, the VTSC producers could generally control the market for nuclear stock production.

5.4 Use of micropropagation methods to minimise contamination

In the last few years, techniques have been developed to produce large quantities of disease-free plants *in vitro* in the laboratory, over short periods of time. Regarding potatoes, the publication by Roca, Espinosa, Roca and Bryan (1978) illustrates the method. In Scotland, it is thought that growers using the technique themselves could theoretically produce enough material from a single tuber in 6 months to plant up to 40 hectares (Harper, personal communication). The techniques are not difficult and it is believed that many growers could easily provide themselves with the necessary equipment and acquire the necessary expertise. It is possible that such growers would have to be licenced by DAFS to carry out the process, and methods, equipment and produce inspected by DAFS staff, to comply with the statutory requirements of the Potato Classification Scheme.

The introduction of micropropagation is likely to be the next major step forward in the production of healthy seed potatoes in Scotland. It would certainly lead to better control of *Erwinia* infection, because rapid propagation would avoid the need to gradually build up stocks over the years by multiplication in the field, where they are or may be exposed to pathogens.

5.5 Advisory recommendations to reduce blackleg and soft rot

From the foregoing description of the ecology of the pathogens and the epidemiology of the diseases, it is possible to put forward some relatively simple advice for growers and merchants to reduce blackleg and soft rot incidence. These suggestions have been or will be incorporated into advisory leaflets and other publications, mainly those issued by the three Scottish Colleges of Agriculture.

The advice may be summarised as follows (these items are not in any order of importance):

1. Keep storage conditions dry, and dry tubers as quickly as possible after harvest, especially if the weather at lifting has been wet. Forced draught ventilation is very useful at this time.
2. Chit tubers to give them as good and as early a start as possible.
3. Plant only healthy seed; tubers attacked by fungi and/or bacteria, or suffering from serious physiological disorders must not be used.
4. Clean and disinfect farm machinery and stores as far as possible.
5. Use fertilizers at recommended rates.
6. Do not pulverise haulm. Destroy haulm with a fast-acting desiccant.
7. Lift as carefully and early as possible.
8. Treat irrigation water (with commercially available filters or by ultra-violet light treatment) to reduce or eliminate bacterial infection (especially with high grade stocks, such as VTSC).
9. Maintain a high standard of hygiene on the farm. Do not

accumulate waste potato piles.

10. Roguing to the standard required under the Classification Scheme is imperative in seed stocks. Roguing ware crops is neither necessary nor practical.

11. Tuber chemical treatments to control fungal rots should be considered by each grower, taking into account personal experience in relation to disease occurrence on the farm, and the condition of the tubers. This recommendation is based on the observation that diseased tubers are more likely to give rise to blackleg plants. In the latter case, the degree of maturity and the presence of mechanical damage and soft rot at harvest, are critical factors in deciding whether or not to treat. Generally speaking, the higher the grade, the more advantageous chemical treatment would be.

It is recognised that this advice is merely palliative, but nevertheless it is a list of simple, positive and practical steps the grower may take to limit impact of these diseases.

5.6 Further research on the ecology of soft rot Erwinia bacteria

The ecology of the soft rot Erwinia bacteria was reviewed by Perombelon and Kelman (1980).

As far as disease in potato is concerned, it is clear that although a great deal has been learned in 25 years, much still remains to be done. Plainly there is room for the discovery of better bacteriological and serological tests which will consistently detect and distinguish the various bacteria which constitute the soft rot Erwinia group. Methods

also need refining so that small numbers of bacteria in infected tissue can be detected and identified rapidly especially for classification and export purposes. Here fluorescent antibody staining (FAS), enzyme-linked immunosorbent assay (ELISA) and immunodiffusion tests may have a place, and are being studied in various laboratories.

Techniques based on "conventional" serology (as distinct from FAS and ELISA), bacteriophage typing and colicin typing need further development as a basis for detailed studies on bacterial ecology. These methods are all being examined at present.

Much more work needs to be done to explain the very common occurrence of bacteria in water in drains, streams and rivers. It may be that E. carotovora is a "true" aquatic bacterium (that is to say, it is very commonly present in water!), but, even so, some must come down in rain. However as already mentioned, "scrubbing" by rain cannot explain the occurrence of Erwinia in waters at times of year when Erwinia is absent from the atmosphere. It is well known that the bacterial flora of ground waters reflects to an extent the bacterial populations of the soil through which the water percolates, especially if the rainfall has been heavy. Furthermore, it is said that soft rot Erwinia, unlike many other soil bacteria, are sited superficially on soil particles (Kikumoto and Sakamoto, 1970). Thus it may be that soil is the source of the Erwinia. But we know that, except in certain circumstances, these organisms cannot generally be isolated from soil, that is to say agriculturally worked "top" soil (mostly the A horizon),

which contains many aerobic bacteria and other organisms some of which could be inimical to the survival of Erwinia. Little or nothing is known about the possible survival of Erwinia at different levels in the soil profile, except for work by de Mendonca and Stanghellini (1979) with the sugar beet strain of E. carotovora in Arizona, which suggested that the bacteria could only be found at a depth of 12 cm or more below the soil surface. Some workers have added Erwinia bacteria to parcels of soil, which have then been buried at different depths in other soil. For example, Kikumoto and Sakamoto (1972) buried sterilised soil to which E. carotovora had been added, at depths varying between 10 cm and 50 cm - those nearer the soil surface apparently died, while those deeper down survived. However, such experiments cannot reflect the survival in undisturbed soils, because the methods used would greatly affect the physical, chemical and biological conditions in the soil.

It seems that it would be worthwhile to look for the presence of Erwinia in soils of various kinds at different depths, to include both A and B horizons, and where different kinds of agriculture are practised. The significance of the soil depth may be that as it increases various physical and chemical changes take place, including the availability of oxygen, which in turn affects the oxidation-reduction (redox) potential. Generally speaking in soils and in waters the deeper down the lower the concentration of oxygen. Moreover, as far as soils are concerned, there are increases in the concentration of carbon dioxide. As Erwinias are facultative

anaerobes, could there be an ecological niche in the soil profile where the Erwinias can survive because the redox potential, pH, temperature and chemical composition are favourable to them, but not to strongly competitive aerobic bacteria and other aerobic organisms in the top layers of soil? And does the concentration of CO_2 also play a role here? By the same token it would be of value to find out if Erwinias occur at different depths from the surface sediment downwards in streams and rivers. These phenomena are well known to soil and aquatic bacteriologists, whose studies emphasise the need to investigate such factors as chemical composition, pH and redox potential in relation to the nature and size of the bacterial populations. The paper on aquatic bacteria by Collins (1977) brings this out clearly; in certain lakes in the English Lake District, the heterotrophic facultatively anaerobic bacteria outnumbered heterotrophic aerobic bacteria, even at the sediment surface. At depths of 100 cm or more below the sediment surface, large numbers of heterotrophic facultative anaerobes were found, while aerobes had been greatly reduced. However, the paper does not go on to discuss the identity of the bacteria. Regarding soils, the circumstances are even more complex, since, for instance, soil crumbs larger than about 3 mm in radius have no oxygen at their centres - an example of the importance of the principle that soils contain different microsites where different bacteria are favoured (McLaren and Skujins, 1968). One environmental factor which shows less variation as soil depth increases, is temperature. In Scotland, in arable areas, soil very rarely freezes at depths of 30 cm or more.

Likewise, temperatures are more stable in streams, ponds and lakes. Even in severe weather, it is unlikely that water in streams freezes solid, though temperatures can fall as low as 0.5°C. It has been suggested the apparent disappearance of Erwinia in top soils is due to alternate freezing and thawing (eg. Kikumoto and Sakamoto, 1972).

Another aspect which needs explaining is the appearance of large numbers of Erwinia in the rhizosphere of weeds and crop plants during mid August into September. At present, there does not seem to be any obvious explanation for this phenomenon. They may come from the air, but if they do, how can the sharp fall in population levels in September be explained, because air sampling has shown that Erwinias are commonly present in the atmosphere through September into December (Quinn, Sells and Graham, 1980)? However, it is possible that they originated from groundkeepers at least at some sites studied in 1980. The next stage in this research must be to repeat the 1980 experiments, but at sites where potatoes have not been grown for several years, to see if the same phenomenon takes place. Some of these sites should be chosen so that Erwinia-free potatoes would be cropped during that season, and soil and weeds should be tested for the presence of Erwinia before and after planting and thence throughout the growing season. In some cases, the time which has elapsed between one potato crop and another could be as long as seven years. Consequently there are unlikely to be any surviving potato groundkeepers.

Extrapolating further, if Erwinia bacteria survive throughout the season at some distance below the soil surface, their appearance in association with roots of weeds and crop plants in August and September may reflect some kind of physical or biological process that brings bacteria nearer to the surface, or that plant roots grow down to levels where the bacteria are present at that time of year. An examination of the literature on soil physics, chemistry and biology might give a clue to what is happening. Another factor which is likly to be of importance is the age of the plant. In general the number of organisms in the rhizosphere increases with the increasing age of the plant, but after ripening, the effect diminishes (Rovira, 1965). Regarding Erwinia, Kikumoto (1968) found that in Japan rhizosphere populations of various bacteria in Chinese Cabbage (Brassica pekinensis) changed during the growth stages of the plant, and the appearance of E. carotovora could be related to the changes in the populations of the general bacterial flora. It is also possible that Erwinia cells are at undetectable levels in the soil in which the plant roots are growing, but as the plant ages the root exudates preferentially stimulate the growth of Erwinia to detectable levels (Kikumoto and Sakamoto, 1969).

Finally more work needs to be done to discover the distance over which viable aerosol propagules may spread, deposit on potato leaves and then build up populations on the leaves, As explained earlier, some basic work has been done, but it is mainly qualitative and speculative. However, quantitative data on the number of propagules in unit volume of air generated in the open cannot be obtained at

present because no sampler has yet been devised that can sample isokinetically where the velocity of air is varying constantly. The lack of such an instrument is a severe constraint on developments in this area of investigation, but plainly the construction of such a device is extremely difficult. Workers at the Microbiological Research Establishment, Porton Down, have made attempts over many years, so far with no success. Another aspect concerns the numbers of viable organisms which need to be deposited on potato plants to establish infection. Some attempts have been made at this laboratory to find the minimum effective dose by depositing varying numbers of a known sero group of Erwinia carotovora on to leaves of potato plants raised from stem cuttings. Such experiments are, however, far from easy to design and conduct because the minimum effective dose will probably vary according to the age of the plant, meteorological conditions, and other factors. So far, experiments done using young rapidly growing potato plants, and applying various numbers of organisms to leaves in the evening under moist conditions, indicate that the dose falls in the range 4 to 20 bacterial cells. * A great many more tests meed to be done, using various potato varieties and additional strains of E. carotovora and E. atroseptica to gain a clearer insight into this parameter. Without this knowledge, effective separation distances cannot be finally determined.

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* Footnote: These are probably aggregates of cells, and may be better termed propagules.

PART 2

CONTROL OF CERTAIN POTATO TUBER DISEASES BY CHEMICAL TREATMENTS

SECTION 1: CHEMICAL TREATMENTS: THE PERIOD 1955-1965

Introduction

Various chemical substances have been used to control certain diseases of potato tubers for many years. This presentation does not review the whole of the work done on treatments; the paper by Boyd (1972) gives an excellent resumé and the paper by Graham (1972) gives a critical appraisal of problems and methods of storage disease control. Rather it discusses those fungal diseases of special importance to the Scottish Seed Potato trade, because the diseases are liable to develop when the tubers are already in the hands of the buyer, and which therefore cause problems both to the seller, and to the Scottish Department of Agriculture and Fisheries in its role as the certifying authority.

The Part 2 presentation deals with work done over some 25 years, firstly on the use of organomercurial dipping of solutions, and touching on experiments done to find out whether organotin compounds would be acceptable substitutes for organomercurials. Finally it describes the introduction of 2-aminobutane for tuber fungal disease control, and its activity in comparison with thiabendazole (TBZ) and systemic benzimidazole fungicides including thiophanate-methyl and imazalil.

Apart from blight (*Phytophthora infestans*), the major fungal diseases of Scottish seed potatoes at present are gangrene (*Phoma exigua* var. *foveata*) and skin spot (*Polyscytalum pustulans*), and any chemical which could not control both diseases would be unacceptable commercially. There are of course many other diseases, notably dry rot (*Fusarium solani* var. *caeruleum*)

but it has declined in importance since the 1950s, partly at least because many modern potato varieties are comparatively resistant to the disease. Black scurf (Rhizoctonia solani), and common scab (Streptomyces scabies) are other important diseases, but are rarely as damaging as gangrene, skin spot and dry rot. They can be however especially important in the export trade, and in the 1950s and early 1960s South Africa imposed stringent requirements regarding the presence of these diseases in imported material.

With regard to chemical treatments of tubers in general, tecnazene (tetrachloronitrobenzene, TCNB) is still the most widely used potato treatment (Tucker, 1978), but although it was introduced many years ago to control dry rot, its major use is now as a sprout suppressant on seed potatoes. Up to the late 1950s, the most successful fungicides for tuber disease control were the organomercurials, particularly methoxyethyl mercury chloride (MEMC). The commercial use of a solution of this substance to treat quantities of tubers was pioneered by a Sir Thomas A Wedderspoon of Wedderspoon Processes Ltd, who began large scale dipping in the 1930s.

In the late 1940s, Sir Thomas introduced washing tubers to remove soil and any potato eelworm cysts (Globodera spp.). This resulted in a sparing effect on the dipping solution, since soil reacts with and detoxifies the mercurial Investigations of this treatment were mainly done by Boyd and co-workers (Boyd 1960, Boyd and Penna 1967), but similar kinds of experiments were done at Agricultural Scientific Services, mainly to see how and to what extent the treatment controlled Rhizoctonia solani and common scab.

In later years, a great deal of work was done to find substitutes for mercurials to control the common fungal rots also. This arose from

continuing complaints to DAFS regarding the quality of Scottish seed sold to England.

As in the case of blackleg and soft rot, the various stages of the investigations are conveniently presented chronologically. Likewise, the narrative is brief and aims to draw together the various steps in research and development work. Details are given in the full papers at Appendix 1.

1.1 Experiments on the control of Rhizoctonia in export material, particularly to satisfy South African import requirements

The first experiments were aimed at finding compounds other than mercurials (Graham, Foister and Srivastava 1957) but none of the substances tested was as good as organomercurials. A study of the various organomercurials on the market was then undertaken to discover which were the most effective against Rhizoctonia sclerotia. In addition, wetting agents were added to the mercurial solution to increase penetration into large sclerotia (Graham, 1960).

The results showed that MEMC was the most suitable substance when used in solutions containing 100 ppm mercury, and that the addition of wetting agents increased the antifungal action against large sclerotia.

At the same time large scale experiments to study the many facets of tuber treatment with organomercurials were undertaken by myself and colleagues, though the results were never published, partly because some of the data were of a confidential nature at that time. An example of the materials, methods and results of two such experiments is given in Appendix 3.

In the course of the experiments untreated unwashed tubers were compared with washed only and washed and disinfected with MEMC. When these were grown in the field the following year, it was found that blackleg was often much more prevalent in washed only tubers, and, that the development of the disease was only partly checked by subsequent disinfection. This effect was mentioned briefly by Graham (1963b), but the full data were never published. The results are shown in the bar graph (fig. 5). The reason for this increase is not clearly understood, but it may be due to an increase in the inoculum potential in lenticels, following movement of water and bacteria into lenticels as a result of the high pressure washing, (the interaction of inoculum density and environmental conditions on disease expression is described by Aleck and Harrison (1978)). The passage of small particles into lenticels was clearly shown by painting the tubers with carbon black ink before washing. The particles of carbon were clearly seen in sections of the lenticels examined microscopically. In most cases, the particles were intercellular, and reached depths of 1 to 2 mm. A similar experiment, using live bacteria made fluorescent by culturing them on nutrient agar containing a fluorescent brightener (J.T.S.), showed the presence of the bacteria in sections of lenticels examined under the UV microscope (fig. 6).

1.2 Investigations with organotin disinfectants

Because of the increasing concern about the toxicity of mercurials, experiments were done to see if any organotin compound could replace MEMC for control of Rhizoctonia. The compounds tested included both alkyl and aryl tins. Details of the experiments are given in Graham (1964).

In summation, it was found that some organotins were very effective

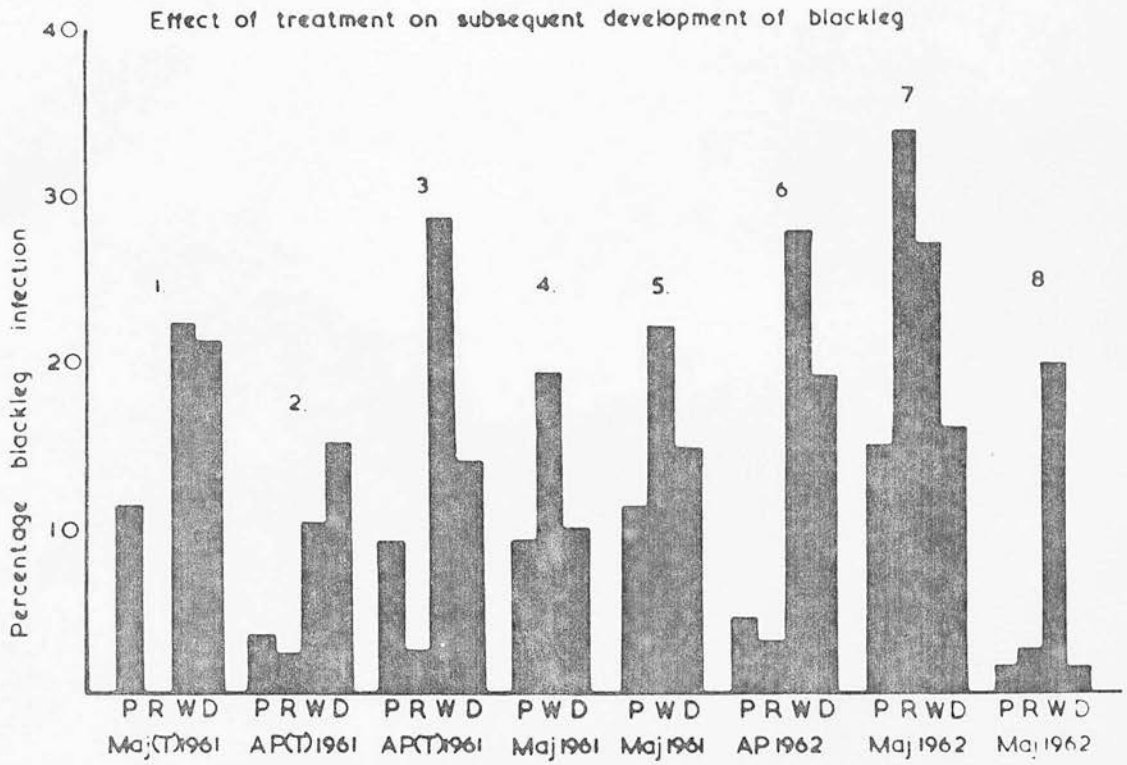

Fig. 5. Bar graph illustrating the effect of tuber treatments on incidence of blackleg in the subsequent crop. Maj = potato variety Majestic; AP = potato variety Arran Pilot ((T) = code for source of tubers). Treatments; P = pitted only; R = riddled and boxed; W = washed only, then boxed; D = washed, dipped in organomercurial then boxed. The blackleg level reached is the sum of several counts during the growing season.

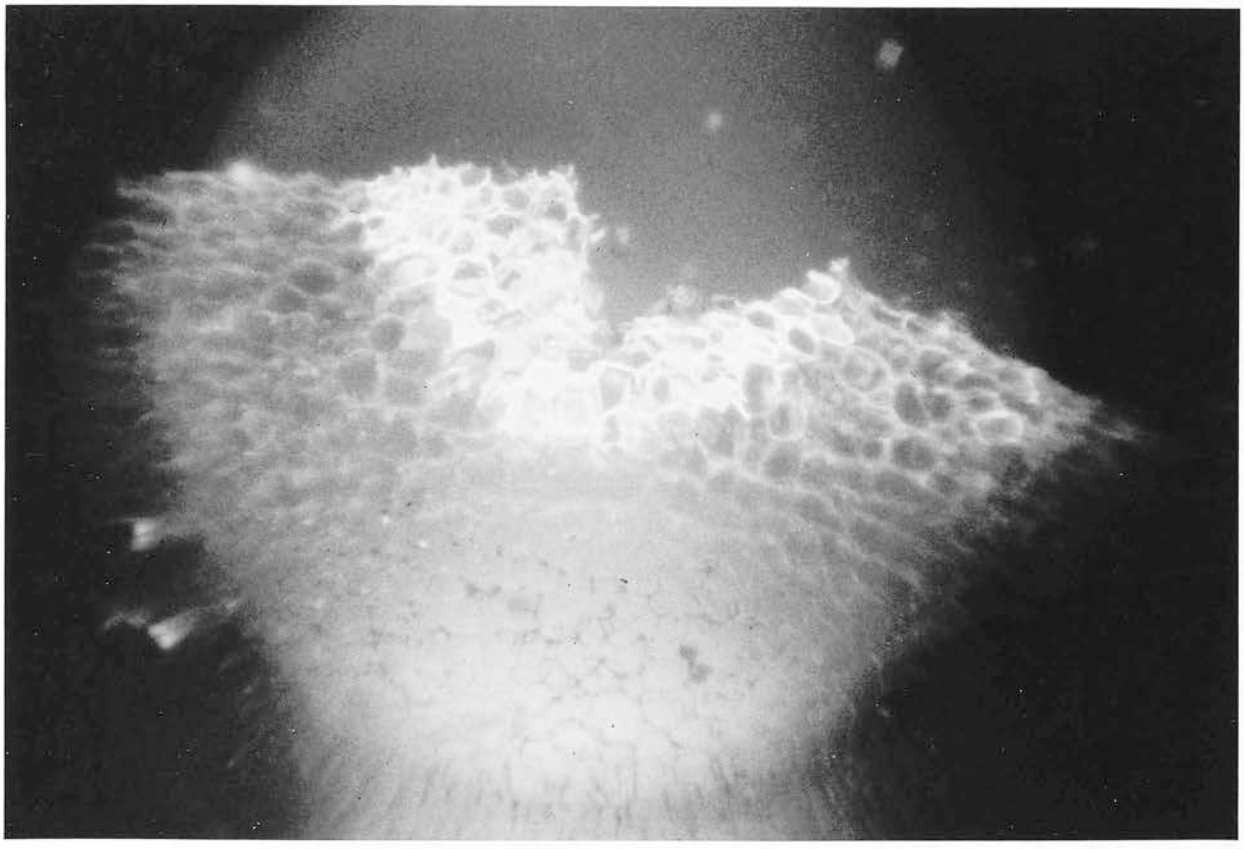

Fig. 6. Photomicrograph (UV) of fluorescence in intercellular spaces of tissue below lenticels; the fluorescence results from ingress of fluorescent cells of Erwinia bacteria.

fungicides; best results were obtained with diethyloctyltin dioxide, but this product was not available commercially. The other, tributyltin acetate, was too phytotoxic to use. It was concluded that no organotin product would be a satisfactory substitute at that time.

SECTION 2: CHEMICAL TREATMENTS: THE PERIOD 1966-1980

2.1 The continuing search for substitutes to organomercury compounds: 2-aminobutane

As already mentioned, because of the toxicity of organomercurials, and also the need to dry tubers after washing, and dipping, together with difficulties in the safe disposal of spent dipping solutions, alternatives were sought. Furthermore, it was considered that avoiding use of water solutions of fungicides was of prime importance, to ensure no increase in bacterial soft rot or blackleg, and therefore it was decided to try to find an antifungal gas or aerosol fog which would penetrate the air spaces between tubers when forced draught ventilation was used.

A number of materials were tried including propargyl bromide, chloropicrin, ammonia, dichlorophen, cyclohexylamine, dibromotetrachloroethane, and 2-aminobutane (sec-butylamine). It was decided to investigate 2-aminobutane in depth, as the first small scale trials showed it to be effective against gangrene and skin spot. Its antifungal properties were first described by Eckert and Kolbezen (1964); the material was very volatile (bp 63°C) and thus easily vaporised, but was highly flammable (flash point, - 19.5°C). The first experiments were done on c. 50 kg of tubers in a small cylindrical chamber (fig. 7). In further tests it was found that 2-aminobutane gave exceptionally good control of gangrene and skin spot (Graham and Hamilton, 1970), so that

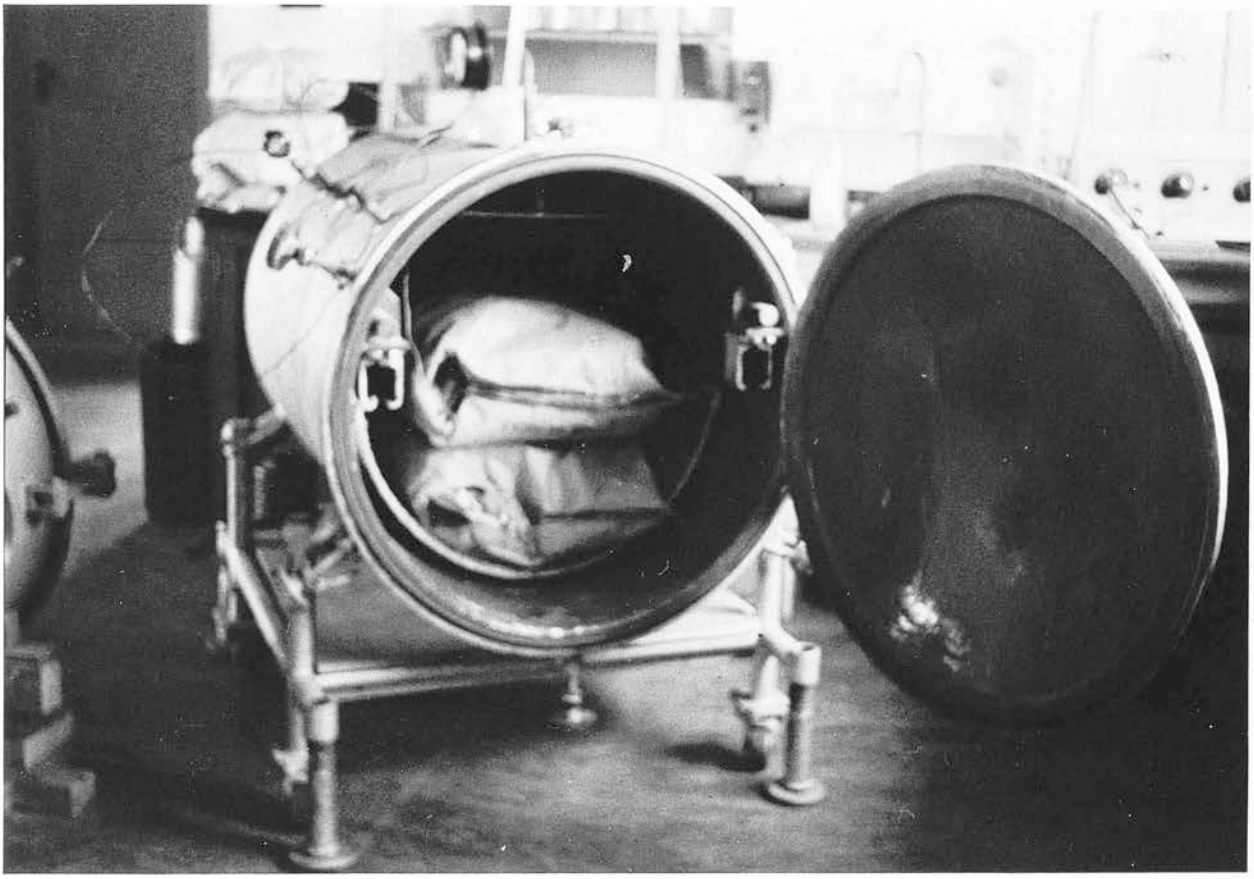

Fig. 7. Small fumigation chamber.

it was decided to build a large experimental fumigation chamber to try to work out the physical methods needed to achieve good control for fumigating a 5 tonne bulk of tubers. This chamber is illustrated in fig. 8. The principles and practice of fumigation of tuber bulks were worked out and published by Graham, Hamilton, Quinn and Ruthven (1973), and the process patented in the United Kingdom and Eire (British Patent Specification No 1268490). The efficiency of the process in controlling diseases is described in Graham, Hamilton, Quinn and Ruthven (1973).

A more sophisticated chamber, designed by the Scottish Institute of Agricultural Engineering, was installed by DAFS at Ingraston farm to fumigate $\frac{1}{4}$ tonne boxes of nuclear VTSC tubers. The chamber is illustrated in fig. 9.

2.2 Development of the 2-aminobutane fumigation process for commercial use

Nothing was known about how the process could be developed in commercial practice and in company with my colleagues we embarked on a series of experiments to discover how to carry out the process most efficiently in farm situations or in large bulks in stores. In addition to the 1973 paper mentioned above, details of bulk fumigations in bins fitted with force draught ventilation and recirculation systems are given in the paper by Graham, Hamilton, Nash and Lennard (1973). The efficacy of treating tubers late in the storage period for control of gangrene was investigated by Graham, Hamilton, Quinn and Ruthven (1975). The effect of treatment on yield was described by Quinn, Harper and Graham (1976).

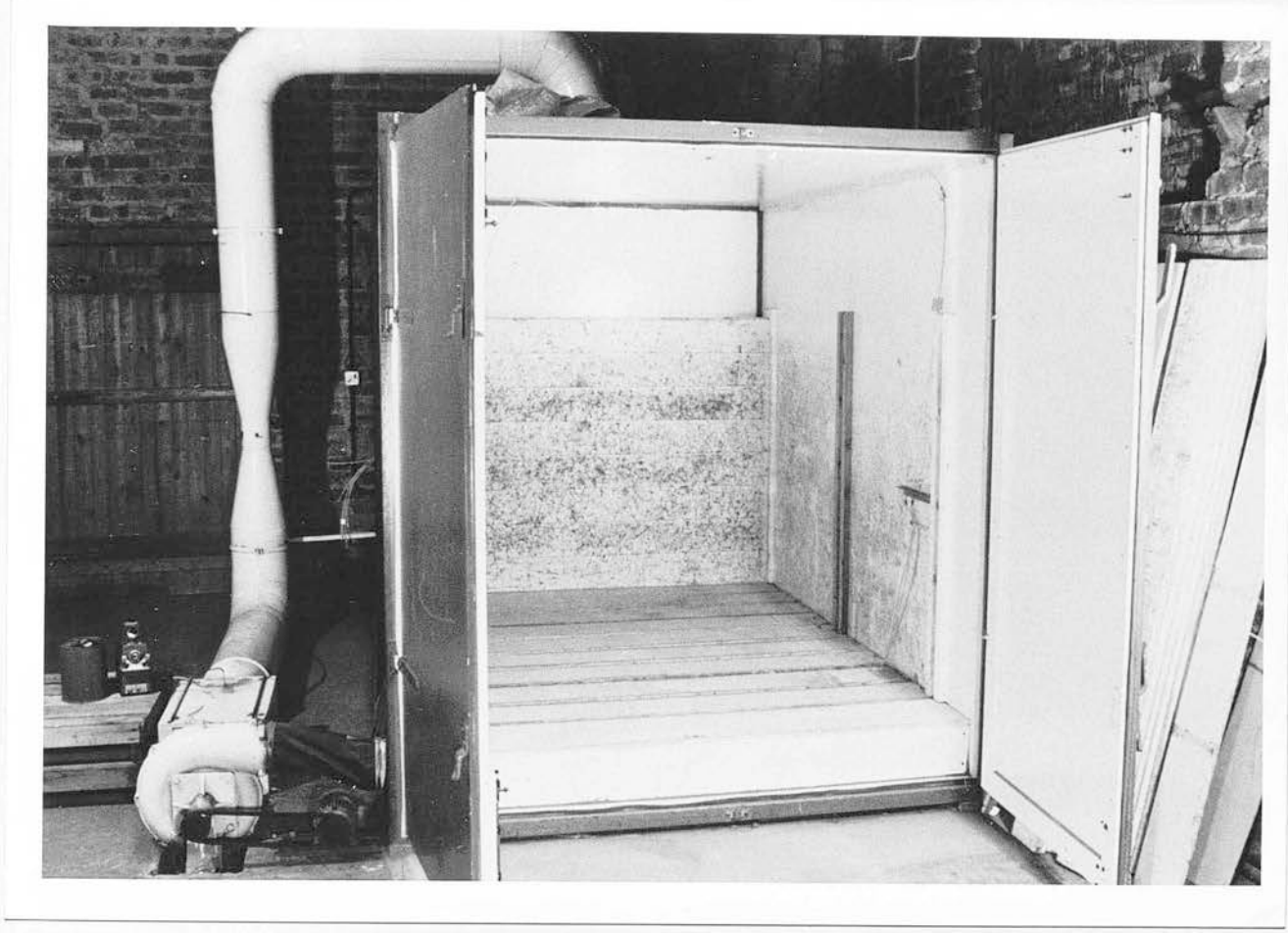

Fig. 8. Experimental 5-tonne fumigation chamber.

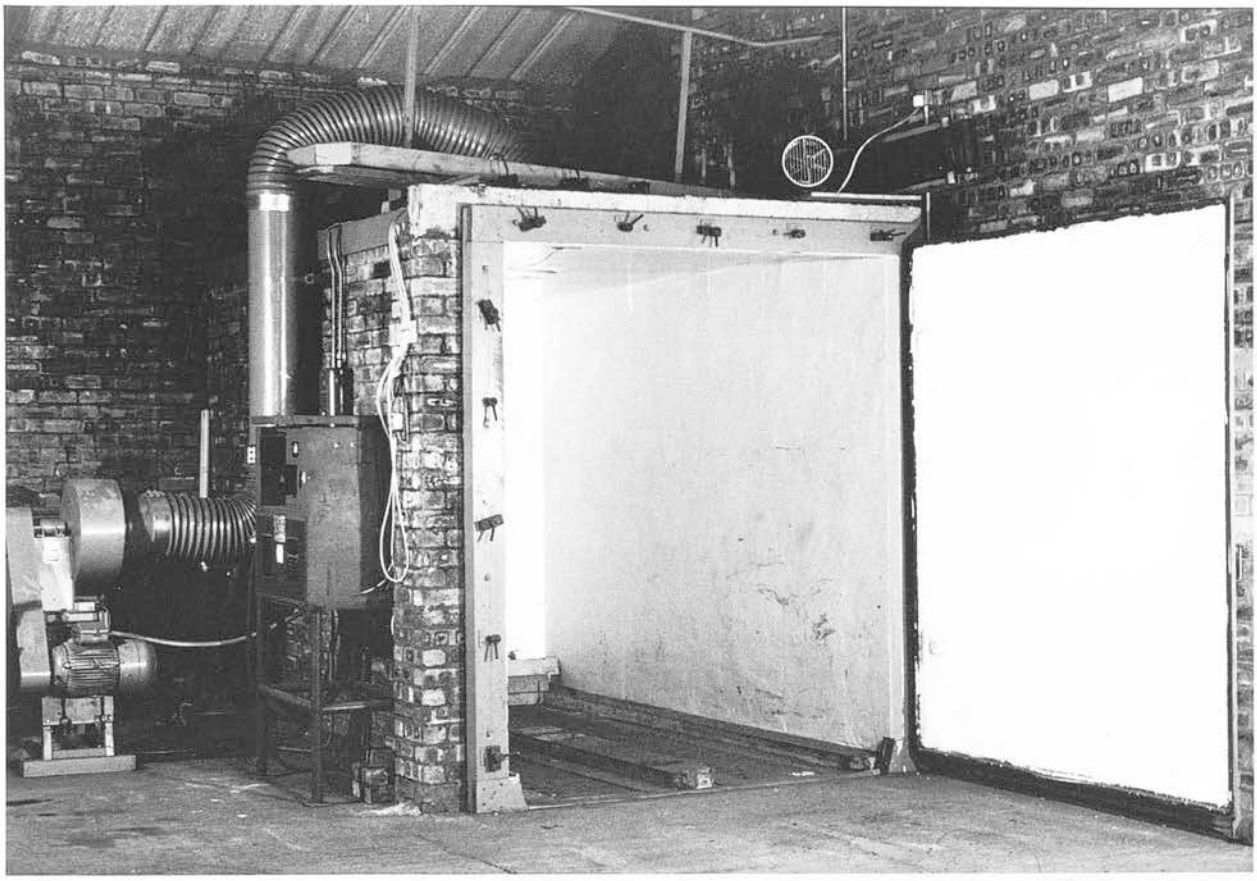

Fig. 9. Ingraston fumigation chamber. Designed by SIAE.

This group of papers formed the basis on which a commercial fumigation procedure was introduced. In summation, research and development work demonstrated the following.

A. That 2-aminobutane fumigation gave very good control of gangrene and skin spot if tubers were treated at a dosage of 200 mg/kg within 14 days of harvest (now extended to 21 days).

B. That it was possible to build fumigation chambers cheaply and efficiently.

C. Force draught ventilation was necessary to achieve good distribution of the gas through the bulk of tubers, because tubers adsorbed 2-aminobutane as it passed from the bottom to the top of a stack. It was found empirically that if the dose was applied within $\frac{1}{2}$ h and then air recirculated for a further 2-2$\frac{1}{2}$ h, much of the adsorbed 2-aminobutane desorbed then resorbed in such a way that residue levels became equilibrated through the stack, which could be up to 3.0 m high. (This latter observation was made using a tall cylindrical fumigation chamber illustrated in Graham, Hamilton, Quinn and Ruthven (1973).

D. Treatment did not control late blight (*Phytophthora infestans*), dry rot (*Fusarium solani* var. *caeruleum*) nor kill the sclerotia of *Rhizoctonia solani*, but there was some control of silver scurf (*Helminthosporium solani*).

E. It was possible to fumigate large bulks in bin storage (around 35-40 tonnes) with suitable modifications of ventilation systems (including the fan). Details are given in the paper by Graham, Hamilton, Nash and Lennard (1973). Fumigation influenced disease control more than any other storage treatment.

F. Fumigation of stocks which had been in storage for several months reduced the amount of further gangrene development if treatment was done soon after grading. However fumigation of tubers with visible symptoms of gangrene did not stop the lesions from spreading (Graham, Hamilton, Quinn and Ruthven, 1975).

G. Field trials to determine any effect of fumigation on subsequent growth and yield from tubers were done over the period 1968 to 1973. The 13 experiments showed that fumigation did not have any substantial effect on growth pattern of yield, although in most cases there were small increases in the number of sprouting eyes, percentage emergence, stem number and in weight and number of seed tubers at harvest. These differences rarely reached statistically significant levels (Quinn, Harper and Graham, 1976).

H. Heavily damaged or immature tubers could not be treated, as the 2-aminobutane entered the exposed living tissue and caused chemical damage which disfigured the tubers.

I. The use of 2-aminobutane in the various situations was not hazardous to operators.

SECTION 3: CLEARANCE FOR SAFETY AND APPROVAL OF EFFICACY UNDER OFFICIAL SCHEMES, AND PATENTING

3.1 Procedure for obtaining safety clearance for use of 2-aminobutane under the terms of the official Pesticides Safety Precautions Scheme (PSPS)

As 2-aminobutane was not then in use as a fungicide, but as an industrial solvent and plasticizer, steps had to be taken to obtain safety clearance under the terms of the official PSPS. Much detail is needed so that the Advisory Committee on Pesticides can form an opinion about safety of chemicals with regard to operators, consumers, captive animals and wildlife and the environment generally. The data required includes information on the chemical and physical properties of the substance, various toxicological data, residue levels in treated produce and any foodstuff derived from treated produce, and many other aspects. This compilation is known as the Summary Data Sheet. (All the requirements are set out in the official PSPS booklet, issued on behalf of several Government Departments by MAFF, 1979).

In order to comply, a Data Sheet was prepared by DAFS staff - this is reproduced at Appendix 4. However, as no Government Department can itself notify a chemical to PSPS, the chemical companies interested in the use and marketing of the chemical (namely BASF and Shell) were supplied with the Data Sheet, with which they notified the chemical to PSPS.

Since 2-aminobutane is a fumigant, the PSPS requires an official document to be issued, giving instructions of the safe use of the chemical to those applying it and to the produce. The document, in this case called "The Safe Use of 2-aminobutane for Fumigation of Potatoes", was prepared by my colleagues and myself and issued by MAFF and DAFS. The latest edition is reproduced at Appendix 5. On the basis of the data supplied, both BASF and Shell received clearance for use on seed potatoes, and later for use on both seed and ware up to a bulk of 250 tonnes. The PSPS Safety clearance sheet is reproduced at Appendix 6; (Recommendations for Safe Use No 1292).

3.2 Procedure for obtaining official approval for efficacy of 2-aminobutane in controlling potato tuber diseases through the Agricultural Chemicals Approval Scheme (ACAS)

On the basis of the data concerning the efficacy of the treatment, mostly contained in the various papers mentioned above, BASF sought approval for use of 2-aminobutane under the official Agricultural Chemicals Approval Scheme. This was granted in 1973, and 2-aminobutane appears in the ACAS booklet "Approved Products for Farmers and Growers", issued annually by MAFF.

3.3 Patenting the process through the National Research and Development Corporation (NRDC), and licencing of companies to use the process through NRDC

The process was patented under the NRDC. The official patent document is reproduced at Appendix 7. The NRDC issued licences to carry out

the process to the Chemical Spraying Company Limited, Perth; Wedderspoon Processes Ltd, Forfar; and D Coakley Company Limited, Dublin, Eire. The two Scottish companies have developed the process considerably; both can supply mobile fumigation units or purpose-built fumigation chambers of their own design. Two of these are shown in figs. 10 and 11. Moreover, one company has successfully undertaken fumigation of bulks up to 2,000 tonnes under limited clearance from PSPS and under direction from DAFS. Figs. 12, 13 and 14 illustrate aspects of the method of fumigating large bulks.

3.4 Practical use made of the fumigation process

The use of 2-aminobutane on potatoes has increased considerably in the last two or three years, some 54,500 tonnes, mainly seed tubers, having been treated in the storage period of 1978-79. Much of the VTSC seed produced in Scotland is fumigated to ensure complete freedom of the high grade produce from gangrene and skin spot.

SECTION 4; CHEMICAL TREATMENTS: COMPARISON OF 2-AMINOBUTANE FUMIGATION WITH OTHER CHEMICAL TREATMENTS

Over the years 1966-80, experiments were done comparing the effectiveness of a number of known fungicides and certain other chemical substances for post harvest control of gangrene, skin spot and dry rot. A paper describing this work is in press, a copy of the typescript is given at Appendix 8.

Regarding gangrene and skin spot, generally good control was achieved by fumigation with 2-aminobutane, but there was insufficient dry rot in stocks to judge its efficacy against this

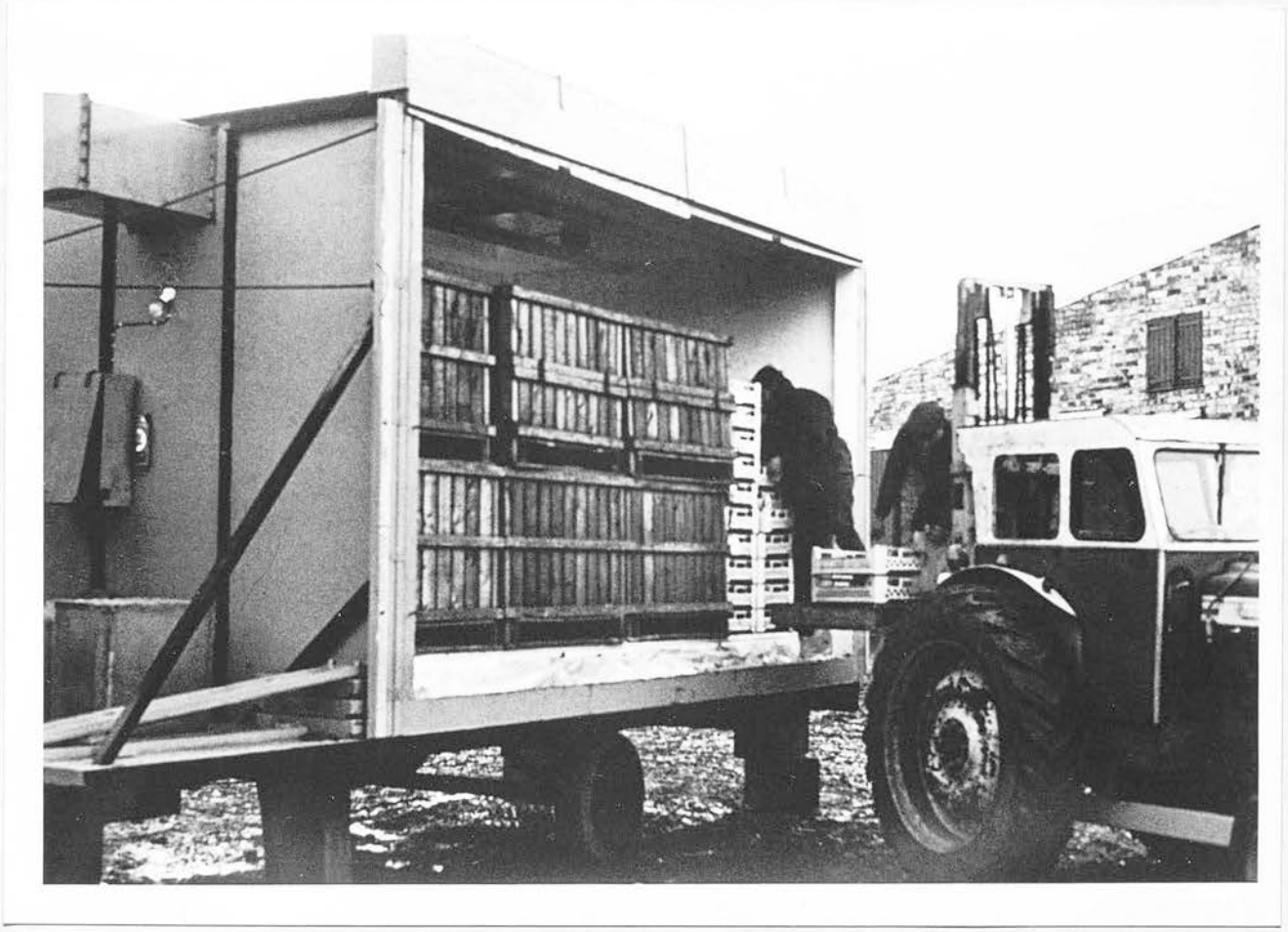

Fig. 10. Commercial mobile fumigation chamber.

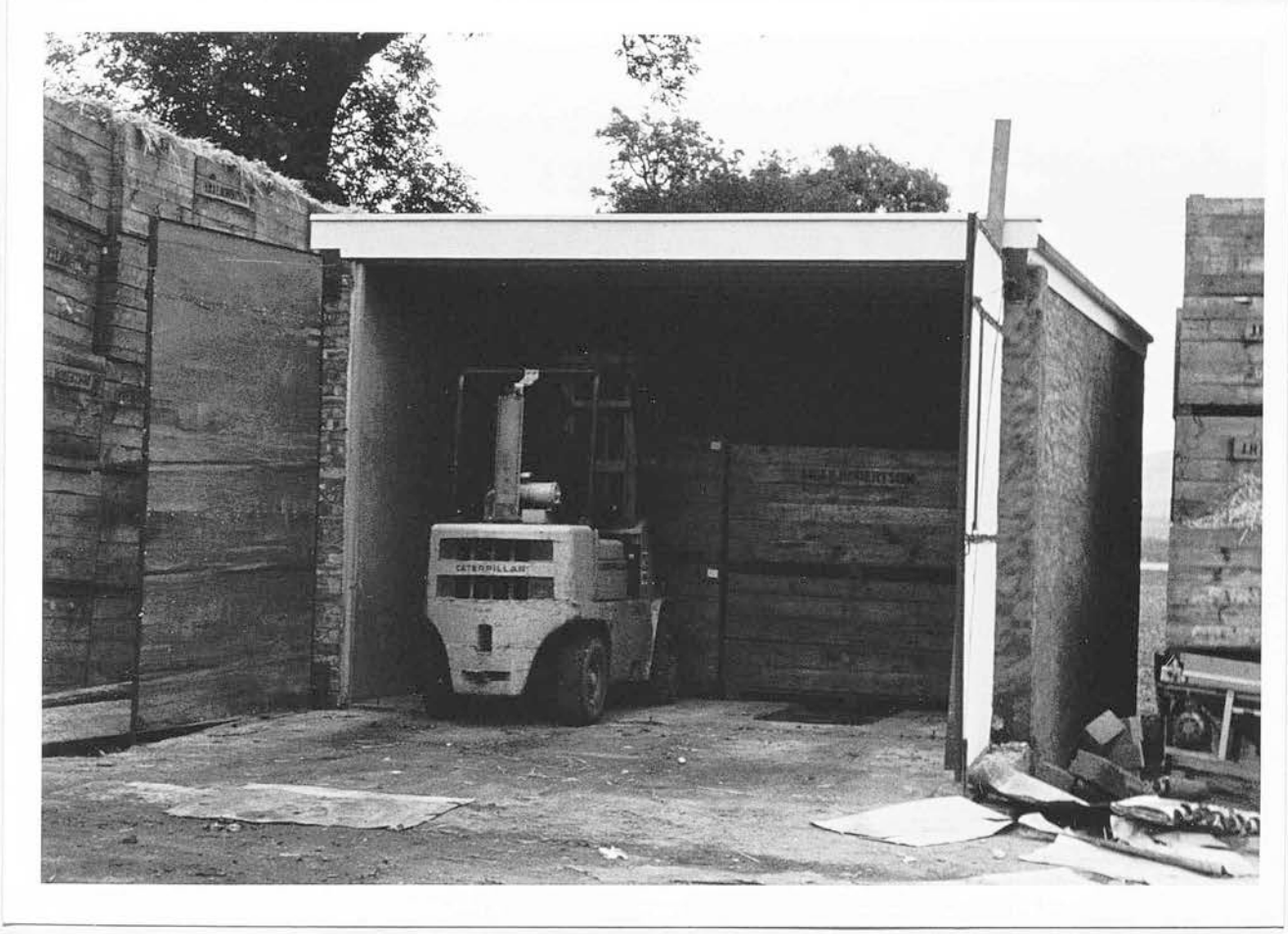

Fig. 11. Commercial 20-tonne fumigation chamber.

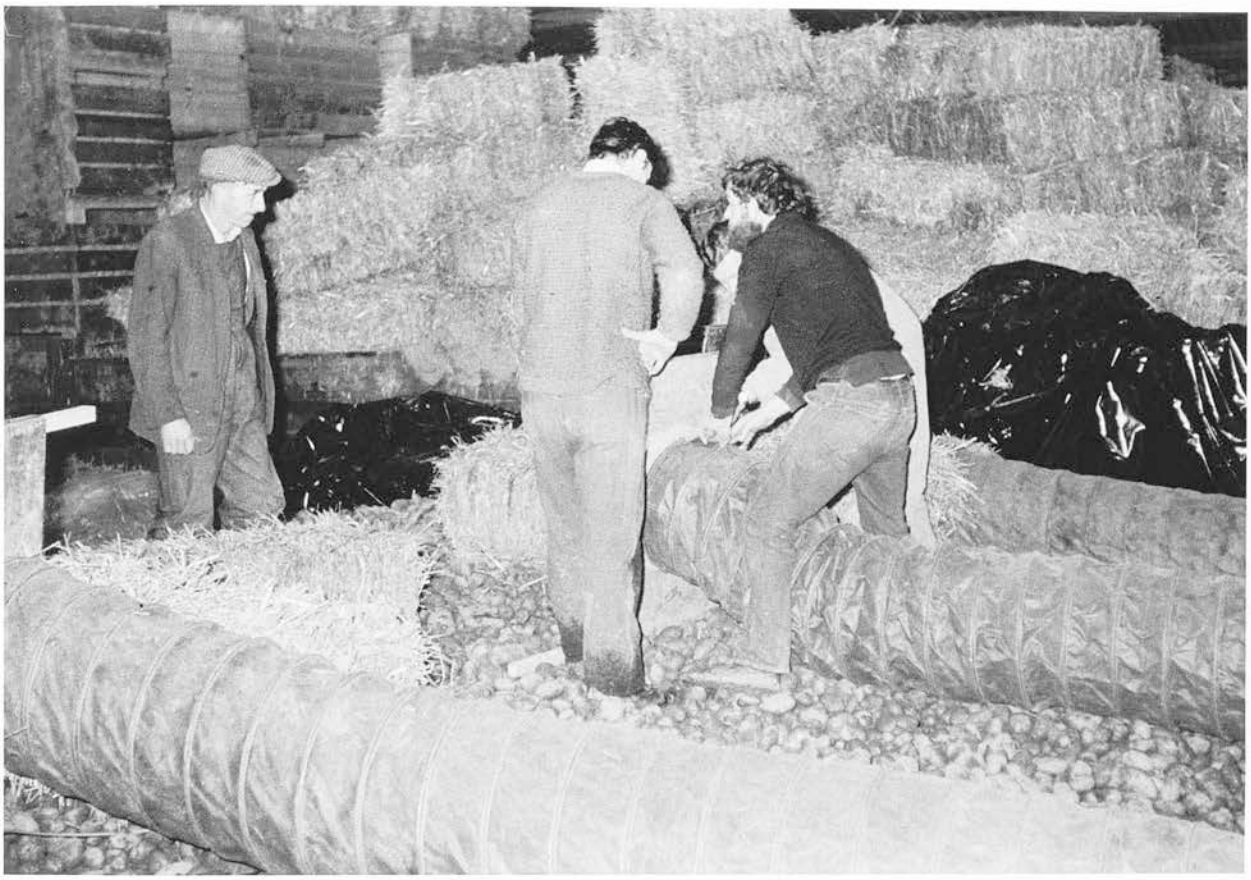

Fig. 12. Laying pipes on surface of large bulk for fumigation.

Fig. 13. Multiple vaporising and fan system for introduction of 2-aminobutane into large bulks.

Fig. 14. Injection system for spraying 2-aminobutane into air ducts for large scale fumigation of bulks.

disease. Dichlorophen fogs and dips were ineffective. Thiabendazole fogs and dips and thiophanate-methyl and imazalil mists did not give as good a control of gangrene or skin spot as 2-aminobutane. However results reported by other workers (eg Boyd, 1977; Cayley, Hide, Lord, Austin, and Davies, 1979) indicate generally acceptable levels of control with thiabendazole mists, which at present is the treatment most commonly used in commerce. The failure to give adequate control in our experiments was thought to be due variously to too low residues (as demonstrated by residue analysis), inability of toxicants to diffuse through tuber skin to reach fungi when they become too deep-seated, and inadequate distribution of the chemicals over the tuber surface. A mist formulation combining thiabendazole and 2-aminobutane was very effective against gangrene, but the formulation proved unstable and could not be marketed commercially.

It is concluded that although there is no chemical product that controls all three diseases well, there are several fungicides whose use can be adapted to particular needs and storage facilities belonging to farmers and merchants.

SECTION 5: CHEMICAL TREATMENTS: FUTURE OUTLOOK

Perhaps not surprisingly, there is considerable room for improvement in both the chemicals used and the methods of application. The principal drawbacks to fumigation are the need to have a fumigation chamber, or to have storage with forced draught ventilation systems

to introduce the gas and recirculate the gas/air mixture through potatoes.

Difficulties regarding inadequate distribution can occur, especially if much soil is present. Moreover, the treatment is necessarily a batch and not a continuous process. Nor does 2-aminobutane control dry rot. In this latter case, my colleagues and I have searched for a volatile substance active against dry rot fungi to admix with 2-aminobutane, but with no success as yet. Substances tested include 1,1,1-trichloroethane, chlorobenzene, bromobenzene, 4-bromoisopropylbenzene, 1,2,3,4-tetramethylbenzene and benzyl isothiocyanate.

Chemical analysis of treated tubers for thiabendazole, thiophanate-methyl and imazalil showed erratic and low residues on many occasions, which largely accounts for poor levels of disease control, and it is in the design and use of applicators that improvements should be sought. Moreover most mist applications introduce water on to the tubers, and not infrequently bacterial soft rot ensues, especially if the lifting season is wet, and autumn temperatures remain high, as in 1978 and 1979.

Another interesting possibility would be to formulate a fogging preparation containing a mixture of 2-aminobutane and thiabendazole that would not be decomposed during fogging. Early attempts by colleagues to formulate a 2-aminobutane fog (not recorded in any of the scientific papers) were a failure owing to decomposition of the

2-aminobutane by the heat generated in the fogging machine ("Swingfog", Jaydon Eng. Co.).

Thinking even further ahead, consideration might be given to the possibility of synthesizing substances which had both an antifungal and sprout suppressant activity, say by combining the aminobutyl-antifungal moiety with the sprout suppressants tecnazene, chlorpropham, or dimethylnaphthalene isomers.* This could be a very valuable step forward for those who store ware potatoes in large bulks for processing, and where sprout suppressants are a vital part of the storage technique. One chemical company is now (1981) attempting to synthesise 1,6-dimethyl-2-(2-aminobutyl) naphthalene with a view to evaluating its physical, chemical, and biological properties.

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*Footnote: The sprout inhibitory action of dimethylnaphthalenes was reported by Meigh, Filmer and Spinks (1973). However they have never been developed commercially, essentially because they have no patent protection.

PART 3

IDENTITY OF CERTAIN ROD-SHAPED, GRAM-NEGATIVE, FLAGELLATED, YELLOW-PIGMENTED FERMENTATIVE BACTERIA ISOLATED FROM PLANTS AND ANIMALS

Introduction

In the general context of the responsibility of Agricultural Scientific Services to identify plant pathogenic bacteria for quarantine purposes and for the Scottish College Advisory Services, studies began in the late 1950s on the identity and properties of certain rod-shaped, gram-negative, flagellated, yellow-pigmented fermentative bacteria which are commonly isolated from plants and seeds, and occasionally from animals.

As pointed out by Graham and Hodgkiss (1967), early bacteriologists were attracted by their colour, and gave them a variety of generic and specific names which led to confusion about the identity of the organisms when studied by medical, general and agricultural bacteriologists, and plant pathologists. As explained by Graham and Hodgkiss (1967), the commonest name at that time was *Bacterium herbicola* Geilinger, but other names such as *Xanthomonas herbicola* Gorlenko, *Pseudomonas trifolii* Huss and *Erwinia lathyri* (Manns and Taubenhaus) Holland, appeared to refer to the same organism. Moreover, there were in the phytopathological literature, similar bacteria said to be associated with plant diseases, including *Erwinia milletiae* (Kawakami and Yoshida). Magrou, *Erwinia ananas* Serrano, *Erwinia uredovora* (Pon, Townsend, Wessman,

Schmitt and Kingsolver) Dye and Erwinia vitivora (Baccarini) du Plessis (Graham, 1958b). The organisms which were isolated by general and medical bacteriologists were first referred to as Bacterium typhi flavum Breed, and later to Chromobacterium typhi-flavum (Breed) Wilson and Miles. In 1965 Muraschi, Friend and Bolles (1965) described similar bacteria isolated from the internal organs of deer and from human throats, stating that they resembled Erwinia milletiae a culture of which they had obtained from the American Type Culture Collection.

To try to clarify the properties, relationships and identity these bacteria, cultures labelled E. lathyri, E. milletiae, E. uredovora and E. ananas, B. herbicola, B. typhi flavum, and the organisms from human throats and organs of deer isolated by Muraschi, Friend and Bolles (1965), were received from various sources and examined bacteriologically. (E. vitivora cultures were not available at that time.)

1.1 Characterization and identification of the bacteria as Erwinia herbicola

The collection of bacteria (35 cultures) was examined for morphology, cultural and biochemical properties as described by Graham and Hodgkiss (1967). The results strongly suggested that B. herbicola, B. typhi flavum, E. lathyri, and E. ananas should be classified as Erwinia herbicola (Duggeli) Dye. As there was doubt about the plant pathogenicity of E. milletiae and E. uredovora, it was decided to leave them as separate species.

1.2 Further developments since 1967

De Ley (1968) determined the DNA base composition of 34 out of the 35 strains described by Graham and Hodgkiss (1967). The results were in general agreement with the phenotypic study by Graham and Hodgkiss (1967); however, the *E. ananas* isolates had a base composition on the low side, and likewise *E. uredovora* had a low composition and differed phenotypically also, so that it was suggested they may be a separate species.

Ewing and Fife (1972) published an extensive study of yellow-pigmented rods from many sources, referable to *E. herbicola*. They argued in some detail that the generic name be altered to *Enterobacter*, and the specific name to *agglomerans*, namely *Enterobacter agglomerans* (Beijerinck) Ewing and Fife. However, this nomenclature has never been commonly accepted, and in the eighth edition of Bergeys Manual, 1974, these organisms were placed in the genus *Erwinia* as *E. herbicola* (Löhnis) Dye; *E. ananas* was given varietal status, while *E. uredovora* was retained as a separate species.

1.3 Identity of yellow-pigmented Enterobacteriaceae phenotypically different from Erwinia herbicola

Graham and Hodgkiss (1967) mentioned the occurrence of yellow coliform bacteria not referable to *E. herbicola*, since their phenotypic characters were clearly distinct. One such organism was NCTC 8155, isolated from a can of dried milk and received as *Enterobacter cloacae*. This organism and a number of other similar bacteria from various sources have now been shown to be a distinct group of organisms, named *Enterobacter sakazakii* by Farmer, Asbury, Huckman and Brenner (1980).

1.4 Identity of Agrobacterium gypsophilae Strains NCPPB179 and NCPPB 1948

In 1933, Brown (1934) isolated a yellow pigmented organism which she referred to as Bacterium gypsophilae, from galls on the plant Gypsophila paniculata. Since it was said to form galls on infected plants, it was transferred to the genus Agrobacterium as A. gypsophilae (Brown) Starr and Weiss (Starr and Weiss, 1943). De Ley, Bernaerts, Rassel and Guilmot (1966) studied the DNA base composition of these two organisms. They pointed out that their composition was quite different from that of other species of Agrobacterium, and that they might be related to Erwinia.

At Prof. De Ley's request, my colleagues and I studied the phenotypic characters of the bacteria (including plant pathogenicity). NCPPB179 is considered to be the original isolate of B. gypsophilae referred to by Brown. NCPPB1948 is the organism isolated from galls on roses by Maas Geesteranus and Barendsen (1966). Details are given in the paper by Graham and Quinn (1974).

To summarise, results showed that both strains were morphologically, biochemically and serologically indistinguishable from E. herbicola. However, they were not pathogenic to Gypsophila paniculata and a number of other members of the Caryophyllaceae.

In the eighth edition of Bergeys Manual (1974), A. gypsophilae is given as a synonym of E. herbicola.

In the paper by Graham and Quinn (1974), it was argued that the first person to validly publish the specific name herbicola was not Geilinger in 1921, but Löhnis in 1911. Hence the authorities for the name Erwinia herbicola are (Löhnis) Dye, which is recorded in the Approved

Lists of Bacterial Names (International Journal of Systematic Bacteriology, 30, p. 294, 1980).

1.5 A gall-forming isolate of Erwinia herbicola

In 1979, an isolate of E. herbicola was received from Dr Miller, Plantenziektenkundige Dienst, Wageningen, Netherlands. The isolate was pathogenic on Gypsophila paniculata, forming canker-like galls. This has been confirmed at Agricultural Scientific Services (fig. 15) and as a result of further tests, the organism has been firmly identified as a strain of E. herbicola. A paper describing this isolate is in preparation by Miller, Quinn, and Graham. The mechanism whereby the organism induces cankerous galls is unknown, but might well repay close study, for (by analogy with other pathogens) the pathogenicity may be related to the presence of plasmids which confer virulence on the organism.

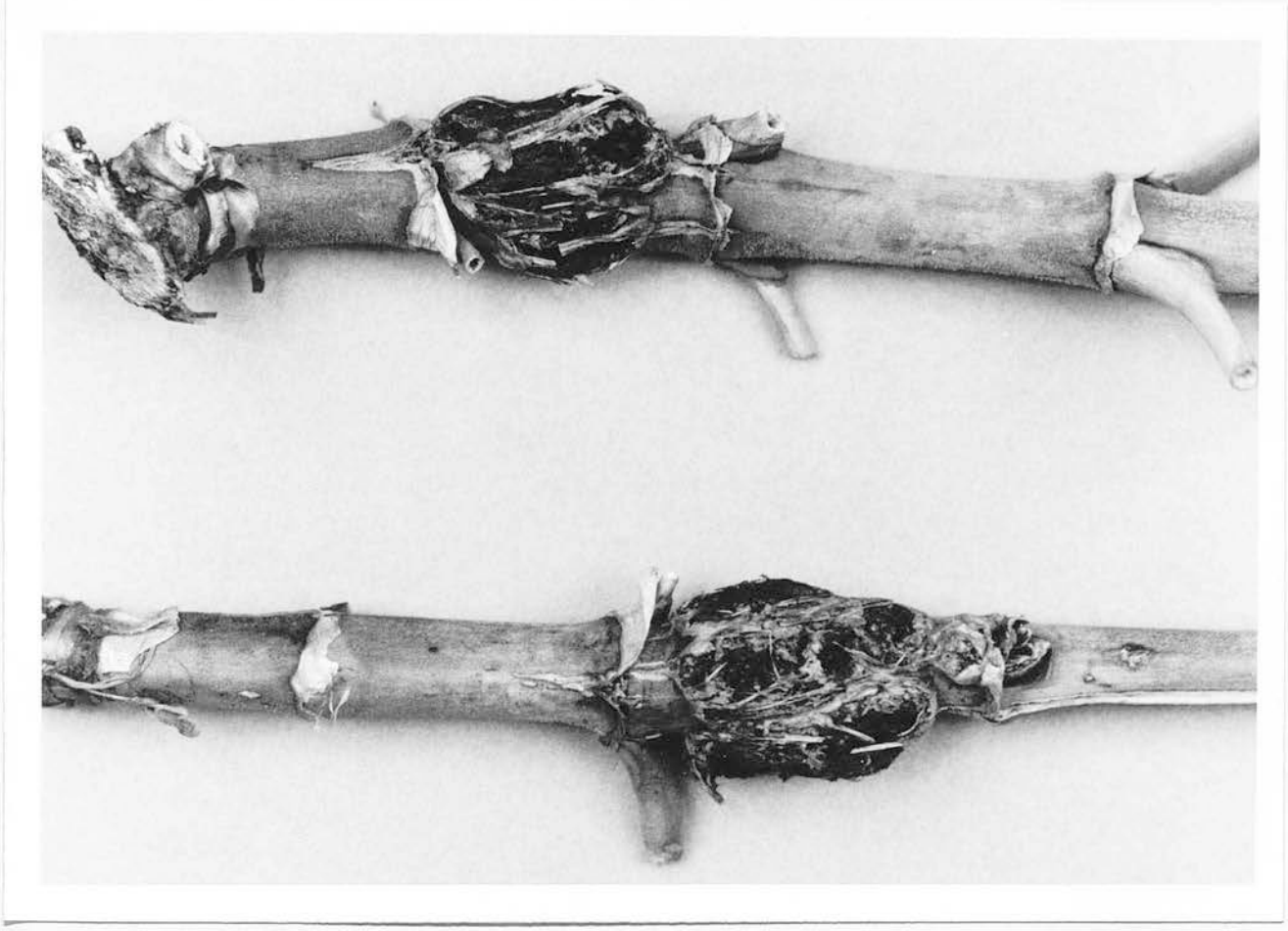

Fig. 15. Canker-like galls on Gypsophila paniculata.

CONCLUDING REMARKS

There can be no doubt that the ecology of potato pathogens, disease epidemiology and control methods have become much better understood during the past 25 years. But in spite of these developments, losses caused by the various diseases still remain high. There is no simple answer to this problem; indeed the reasons why disease continue to take their toll are many and complex, even with respect to each separate disease and the causal organism.

Sometimes failure may be due to growers not abiding by simple common-sense advice, but on the other hand, it must be admitted that a great deal of advice is gratuitous in the sense that the circumstances during each growing and harvesting season vary so much. An example of this is the statement that potatoes should be "cured" after lifting, to heal wounds quickly and prevent fungal attack, "unless the crop may develop soft rot". In many cases, it is not known whether soft rot is liable to develop, and no satisfactory simple tests for "rotting potential" have been found yet. If seed is kept cold to prevent sprouting during storage, the diseases favoured by cold conditions, such as gangrene, may develop. On the other hand, where tubers are stored comparatively warm, as happens with potatoes for processing, diseases favoured by higher temperatures, such as silver scurf, may develop instead. In addition, it must be recognised that storage conditions are largely dictated by the need to control sprouting, and in other circumstances to provide tubers having the correct chemical composition rather than to achieve disease control as in the case of potatoes for processing. These points were made in the paper by Graham, (1972), where it was also argued that the answer lay in producing tubers

essentially free from pathogens before storage. At that time it was thought possible that a combination of stem cutting propagation and chemical treatments could produce large quantities of essentially pathogen-free material, largely by a process in some ways analagous to dilution, in that a continuous introduction of pathogen-free stocks into commerce from the nuclear stock farm would gradually dilute and displace diseased material. However, it must be said that certain growers and scientists did not see these methods as the panacea for all disease problems that some expected. In the light of further experience it has become clear that, for many reasons, high grade stocks can on occasion become rapidly infected with pathogenic bacteria and fungi despite all reasonable precautions by farmers, although it must also be admitted that at least some infections probably result from poor hygiene.

There seems little doubt that rapid micropropagation of quantities of disease-free material will greatly help by reducing the number of years of exposure to pathogens during the VTSC multiplication phase, but even so, (as the ecology of the pathogens is becoming better understood) success in keeping stocks free from such a widely distributed organism as Erwinia carotovora is likely to be limited. These kinds of pathogens could only be combatted by chemical treatments, but as mentioned earlier, no satisfactory chemical is known at present. On the other hand, E. atroseptica, the pathogen which causes blackleg in Scotland, is not so common in the environment, and hence a useful degree of control has been achieved (Perombelon, Lowe, Quinn and Sells, 1980). Iodophor fogs have not controlled soft rot successfully (this statement is derived from personal observations made on commercial treatments with iodophor and is not based on experiments); and whereas there is evidence that fumigation with chloropicrin can destroy Erwinia within tubers

(Kloepper, Schroth, Weinhold and Bowman, 1979) this substance is so toxic both to plants and animals, it could never be used commercially. At present tests are being done at Agricultural Scientific Services on a mixture of 8-hydroxyquinoline and thiabendazole to control tuber bacterial soft rots and fungal rots.

Prospects for better control of fungal diseases by application of newer systemic fungicides such as imazalil, sisthane, nuarimol and prochloraz are promising (Hyde and Cayley, 1980), and whereas present methods of integrated control of fungal diseases have their limitations, it cannot be denied that tuber health has generally improved over the past decade. It is certain that the potato trade would never wish to abandon the multiplication of stocks from pathogen-free material.

Finally, turning to the bacteriology and ecology of bacteria presently encompassing the genus Erwinia, there is a great deal still to be learned. For one thing, the genus as present constituted, is made up of organisms whose bacteriological, serological and pathogenic properties vary a great deal. In this connection, the work done on the yellow-pigmented organisms making up the species Erwinia herbicola illustrates the point. These bacteria are ubiquitous, and they are associated with pathogenesis in human beings, animals and plants, although most isolates appear to be saprophytes.

The soft rot Erwinia bacteria pose very great problems of pathogenesis and epidemiology. This arises from relative lack of specificity in their host range, their relatively simple demands on food sources and their tolerance to a wide range of temperatures. It would be very interesting to study their occurrence and distribution in tropical environments using the techniques developed for study developed in cool temperate regions, and especially, perhaps, their occurrence in rivers and in the atmosphere. One wonders what could be found, for example, in the river Amazon

AKERS, T. G. et al. (1979). Factors in the production, release and viability of biological particles. Bacteria and viruses. In "Aerobiology" ed. Robert L. Edmonds, pp 11-22. Dowden Hutchinson & Ross Inc.

ALCOCK, N. L. & FOISTER, C. E. (1936). A fungus disease of stored potatoes. Scot. J. Agric. 19, 252-257.

ALECK. J. R. & HARRISON, M. D. (1978). Influence of inoculum density and environment on the development of potato blackleg. Amer. Potato J., 55, 479-494.

ANDERSEN, A. H. (1958). New sampler for the collection, sizing and enumeration of viable airborne particles. J. Bact. 76, 471-484.

BOYD, A. E. W. (1960). Fungicidal dipping and other treatments of seed potatoes in Scotland. Eur. Potato J. 3, 137-154.

BOYD, A. E. W. (1972). Potato Storage Diseases. Rev. Pl. Path., 51, 297-321.

BOYD, A. E. W. (1974). Sources of potato blight (Phytophthora infestans) in the east of Scotland. Plant Path. 23, 30-36.

BOYD, A. E. W. (1977). Control of certain potato tuber diseases by thiabendazole and 2-aminobutane. Proc. Symp. on Pest and Disease Control in Northern Britain; Scottish Hortic. Res. Inst., pp. 42-44.

BOYD, A. E. W. & PENNA, R. J. (1967). Seed tuber disinfection. Proc. 4th Brit. Insectic. and Fungic. Conf. 1, 294-302.

BRENNER, D. J., FANNING, G. R., & STEIGERWALT, A. G. (1977). Deoxyribonucleic acid relatedness among Erwiniae and other enterobacteria. II Corn stalk rot bacterium and Pectobacterium chrysanthemi. Int. J. Syst. Bact. 27, 211-221.

BROWN, N. A. (1934). A gall similar to crown gall, produced on Gypsophila by a new bacterium. J. Agr. Res. 48, 1009-1112.

BURR, T. J. & SCHROTH, M. N. (1977). Occurrence of soft rot Erwinia spp. in soil and plant material. Phytopathology 67, 1382-1387.

CAYLEY, G. R., HIDE, G. A., LORD, K. A., AUSTIN, D. J. & DAVIES, A. R. (1979). Control of potato storage diseases with thiabendazole. Potato Res. 22, 171-190.

CHAMBERLAIN, A. C. (1966). Transport of gases to and from grass and grass-like surfaces. Proc. Roy. Soc. A, 290, 236-265.

CHAMBERLAIN, A. C. (1967). Deposition of particles to natural surfaces. In "Airborne Microbes", 17th Symposium of Soc. Gen. Microbiol., ed. Gregory and Monteith, pp. 138-164. Cambridge Univ. Press.

CHATTERJEE, A. K. & STARR, M. P. (1980). Genetics of Erwinia spp. Ann. Rev. Microbiol. 34, 645-676.

COLLINS, VERA G. (1977). Methods in sediment microbiology. Advances in Aquatic Microbiology 1, 219-272.

CUPPELS, D. & KELMAN, A. (1974). Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology 64, 468-475.

DE BOER, S. H., COPEMAN, R. J. & VRUGGINCK, H. (1979). Serogroups of Erwinia carotovora potato strains determined with diffusible, somatic antigens. Phytopathology 69, 316-319.

DE LEY, J. (1968). DNA base composition and hybridization in the taxonomy of phytopathogenic bacteria. Ann. Rev. Phytopathol. 6, 63-90.

DE LEY, J., BERNAERTS, M., RASSEL, A. & GUILMOT, J. (1966). An approach to an improved taxonomy of the genus Agrobacterium. J. Gen. Microbiol. 43, 7-17.

DE MENDONCA, M. & STANGELLHINI, M. E. (1979). Endemic and soil-borne nature of Erwinia carotovora var. atroseptica, a pathogen of mature sugar beets. Phytopathology 69, 1096-1099.

DRUETT, H. A. (1973). The open air factor. In "Airborne Transmission and Airborne Infection", ed. Hers, J. H. Ph. & WINKLER, K. C., pp. 141-149. Utrecht. Oosthoek Publishing Co.

DYE, D. W. (1969). A taxonomic study of the genus Erwinia. II. The "carotovora" group. N.Z. J. Sci. 12, 81-97.

ECKERT, J. W. & KOLBEZEN, M. J. (1964). Control of Penicillium decay of oranges with certain volatile aliphatic amines. Phytopathology 53, 1053-1067.

ERINLE, I. D. (1975). Growth of Erwinia carotovora var. atroseptica and E. carotovora var. carotovora in potato stems. Plant Path. 24, 224-229.

EWING, W. H. & FIFE, M. A. (1972). Enterobacter agglomerans (Beijerinck) comb. nov. (the herbicola-lathyri bacteria). Int. J. Syst. Bact. 16, 459-490.

FARMER, J. J., ASBURG, M. A., HICKMAN, F. W. & BRENNER, D. J. (1980). Enterobacter sakazakii, a new species of "Enterobacteriaceae" isolated from clinical specimens. Int. J. Syst. Bact. 30, 569-584.

GRAHAM, D. C. (1958a). Occurrence of soft rot bacteria in Scottish soils. Nature, Lond. 181, 61.

GRAHAM, D. C. (1958b). The status of Erwinia lathyri and related bacteria. Comm. Phytopath. News 4, 49-51.

GRAHAM, D. C. (1960). Control of Rhizoctonia solani on potato by disinfection of seed tubers with organo-mercury compounds. Eur. Potato J. 3, 80-89.

GRAHAM, D. C. (1962). Blackleg disease of potatoes. Scott. Agric. 41, 211-215.

GRAHAM, D. C. (1963a). Serological diagnosis of potato blackleg and tuber soft rot. Plant Path. 12, 142-144.

GRAHAM, D. C. (1963b). Spread of the potato blackleg organism during mechanical washing and disinfection of tubers, and possibilities for its control. Proc. 2nd Triennial Conf. of the European Association for Potato Research, pp. 196-197.

GRAHAM, D. C. (1964a). Taxonomy of the soft rot coliform bacteria. Ann. Rev. Phytopathol. 2, 13-42.

GRAHAM, D. C. (1964b). The use of organo-tin compounds as potato tuber disinfectants, particularly against Rhizoctonia solani. Europ. Potato J. 7, 33-44.

GRAHAM, D. C. (1972). Identification of soft rot coliform bacteria. Proc. 3rd Int. Conf. on Pl. Pathogenic Bacteria 1971, PUDOC, pp. 273-279.

GRAHAM, D. C. (1973). Storage requirements for control of tuber diseases and blemishes. Proc. 5th Triennial Conf. of the European Association for Potato Research, 1972, pp. 47-51.

GRAHAM, D. C. & DOWSON, W. J. (1960a). The coliform bacteria associated with potato blackleg and other soft rots. I. Their pathogenicity in relation to temperature. Ann. Appl. Biol. 48, 51-57.

GRAHAM, D. C. & DOWSON, W. J. (1960b). The coliform bacteria associated with potato blackleg and other soft rots. II. Biochemical reactions of low and high temperature strains. Ann. Appl. Biol. 48, 58-64.

GRAHAM, D. C., SRIVASTAVA, S. N. S. & FOISTER, C. E. (1957). The control of Rhizoctonia solani on potato. Plant Path. 6, 149-152.

GRAHAM, D. C. & HAMILTON, G. A. (1970). Control of potato gangrene and skin spot by fumigation of tubers with sec-butylamine. Nature, Lond. 227, 297-298.

GRAHAM, D. C., HAMILTON, G. A., QUINN, C. E., & RUTHVEN, A. D. (1973). Use of 2-aminobutane as a fumigant for control of gangrene skin spot and silver scurf diseases of potato tubers. Potato Res. 16, 109-125.

GRAHAM, D. C., HAMILTON, G. A., QUINN, C. E. & RUTHVEN, A. D. (1975). Control of potato gangrene by fumigation of tubers with 2-aminobutane after periods of storage. Potato Res. 18, 410-415.

GRAHAM, D. C., HAMILTON, G. A., NASH, M. J. & LENNARD, J. H. (1973). Fumigation of bulk stored potatoes with 2-aminobutane for control of gangrene, skin spot and silver scurf diseases. Potato Res. 16, 234-243.

GRAHAM, D. C. & HARDIE, J. L. (1971). Prospects for the control of potato blackleg disease by the use of stem cuttings. Proc. 6th British Insectic. and Fungic. Conf. 1, 219-224.

GRAHAM, D. C. & HARRISON, M. D. (1975). Potential spread of Erwinia spp. in aerosols. Phytopathology 65, 739-741.

GRAHAM, D. C. & HARPER, P. C. (1966). Effect of inorganic fertilizers on the incidence of potato blackleg disease. Eur. Potato J. 9, 141-145.

GRAHAM, D. C. & HARPER, P. C. (1967). Potato blackleg and tuber soft rot. Scott. Agric. 46, 68-74.

GRAHAM, D. C. & HODGKISS, W. (1967). Identity of gram-negative, yellow-pigmented, fermentative bacteria isolated from plants and animals. J. Appl. Bact. 30, 175-189.

GRAHAM, D. C. & QUINN, C. E. (1974). Identification of Agrobacterium gypsophilae Strains NCPPB 179 and NCPPB 1948 as Erwinia herbicola Int. J. Syst. Bact. 24, 238-241.

GRAHAM, D. C., QUINN, C. E. & BRADLEY, L. F. (1977). Quantitative studies on the generation of aerosols of Erwinia carotovora var. atroseptica by simulated raindrop impaction on blackleg-infected potato stems. J. Appl. Bact. 43, 413-424.

GRAHAM, D. C., QUINN, C. E., & HARRISON, M. D. (1976). Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967-1974, Potato Res. 19, 3-20.

GRAHAM, D. C., QUINN, C. E., SELLS I. ANN, & HARRISON, M. D. (1979). Survival of strains of soft rot coliform bacteria on microthreads exposed in the laboratory and in the open air. J. Appl. Bact. 46, 367-376.

GRAHAM, D. C. & VOLCANI, Z. (1961). Experiments on the control of blackleg disease of potato by disinfection of seed tubers with mercury compounds. Eur. Potato J. 4, 129-137.

GREGORY, P. H. (1961). The Microbiology of the Atmosphere, London, Leonard Hill.

HARRISON, M. D., QUINN, C. E., SELLS, I. ANN, & GRAHAM, D. C. (1977). Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to re-contamination of pathogen-free stocks. Potato Res. 20, 37-52.

HARPER, P. C., BOYD, A. E. W., & GRAHAM, D. C. (1963). Growth cracking and bacterial soft rot in potato tubers. Plant Path. 12, 139-142.

HAY, F. G. (1969). Report on the Marketing of Scotch Seed Potatoes. National Farmers Union of Scotland.

HIDE, G. & CAYLEY, G. R. (1980). Tests of fungicides for controlling gangrene (Phoma exigua var. foveata) and dry rot (Fusarium solani var. caeruleum) and Fusarium sulphureum) on potatoes during storage. Potato Res. 23, 395-403

HIDE, G. A., HIRST, J. M. & GRIFFITH, R. L. (1969). Control of potato tuber diseases with systemic fungicides. Proc. 5th British Insectic. and Fungic. Conf. 2, 310-314.

KIKUMOTO, T. (1968). Ecological studies on the soft-rot bacteria of vegetables. V. The variation of the rhizosphere flora in relation to the development of chinese cabbage. Jubilee Publ. Commemoration 60th Birthday of Prof. M. Sakamoto, pp. 355-365.

KIKUMOTO, T. & SAKAMOTO, M. (1969). Ecological studies on the soft-rot bacteria of vegetables. VII. The preferential stimulation of the soft-rot bacteria in the rhizosphere of crop plants and weeds. Ann. Phytopath. Soc. Japan, 35, 36-40.

KIKUMOTO, T. & SAKAMOTO, M. (1970). Ecological studies on the soft-rot bacteria of vegetables. X. The distribution of soft-rot bacteria in soil aggregates. Ann. Phytopath. Soc. Japan, 36, 207-213.

KIKUMOTO, T. & SAKAMOTO, M. (1972). Ecological studies on the soft-rot bacteria of vegetables. XII. Survival of Erwinia aroideae buried at different depths in field soil. Bull. Inst. Agric. Res. Tohoko Univ. 23, 159-172.

KLOEPPER, J. W., SCHROTH, M. N., WEINHOLD, A. R., & BOWMAN, T. (1979). Chloropicrin fumigation for the control of potato blackleg and soft rot. Amer. Potato J. 56, 468-469.

LAZAR, I. (1972). Serological relationships between the "amylovora", "carotovora" and "herbicola" groups of the genus Erwinia. Proc. 3rd Intern. Conf. on Pl. Pathogenic Bact. 1971, PUDOC, pp. 131-141.

LUND, B. M. (1975). Formation of reducing sugars from Sucrose by Erwinia species. J. Gen. Microbiol., 88, 367-371.

LUND, B. M. (1979). Bacterial soft rot of potatoes. In "Plant Pathogens", ed. D. W. Lovelock. Society for Applied Bacteriology Tech. Ser. No. 12, Academic Press., pp. 19-49.

LUND, B. M. & WYATT, G. M. (1973). The nature of reducing compounds formed from sucrose by Erwinia carotovora var. atroseptica. J. Gen. Microbiol. 78, 331-336.

MAAS GEESTERANUS, H. D. & BARENDSEN, H. (1960). Host plants of Agrobacterium gypsophilae. Neth. J. Pl. Pathol. 72, 231-232.

MAY, K. R. (1967). Physical aspects of sampling airborne microbes. In "Airborne Microbes" 17th Symposium of Soc. Gen. Microbiol. ed. by Gregory & Monteith, pp. 60-80, Cambridge Univ. Press.

MAY, K. R. & DRUETT, H. A. (1968). A microthread technique for studying the viability of microbes in a simulated airborne state. J. Gen. Microbiol. 51, 353-366.

MAY, K. R. & HARPER, G. J. (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. Brit. J. Indust. Med. 14, 287-297.

MCLAREN, A. D. & SKUIJENS, J. (1968). The physical environment of micro-organisms in soil. In 'The Ecology of Soil Bacteria", International Symposium, ed. T. R. G. Gray and D. Parkinson, Liverpool Univ. Press, pp. 3-24.

MEIGH, D. F., FILMER, A. A. E. & SPINKS, E. A. (1973). Endogenous growth regulators of potatoes. ARC Food. Res. Int. Report for 1969-1973, p. 17.

MEISLEY, J. C. & STANGELLINI, M. E. (1976). Isolation of soft rot Erwinia species from agricultural soils using an enrichment technique. Phytopathology 66, 367-370

MOUSTAFA, F. A. & WHITTENBURY, R. (1969). A comparison of some phytopathogenic pseudomonads. Phytopath. Z. 67, 63-72.

MURAOCHI, T. F. FRIEND, M. & BOLLES, D. (1965). Erwinia-like micro-organisms isolated from animal and human hosts. Appl. Microbiol. 13, 128-131.

NOBLE, M. & GRAHAM, D. C. (1956). A selective medium for the isolation of coliform soft rot bacteria. Nature, Lond. 178, 1479-1480.

NOBLE, M. & MARSHALL, M. (1952). A note on blackleg of potato. Plant Path. 1, 134.

PASQUILL, F. (1974). Development of practical systems for calculating the concentration pattern from a single source. In "Atmospheric Diffusion", pp. 365-380. Chichester. Ellis Horwood Ltd.

PEROMBELON, M. C. M. (1972). The extent and survival of contamination of potato stocks in Scotland by Erwinia carotovora var. carotovora and Erwinia carotovora var. atroseptica. Ann. Appl. Biol. 71, 111-117.

PEROMBELON, M. C. M. (1978). Contamination of foliage by Erwinia carotovora. Annu. Rep. Scottish Hort. Res. Inst. p. 87. (With R. Lowe).

PEROMBELON, M. C. M., Fox, R. A. & LOWE, R. (1979). Dispersion of Erwinia carotovora in aerosols produced by the pulverization of potato haulm prior to harvest. Phytopath. Z. 94, 249-260.

PEROMBELON, M. C. M. & KELMAN, A. (1980). Ecology of the soft rot Erwinias. Annu. Rev. Phytopathol. 18, 361-387.

PEROMBELON, M. C. M., LOWE, R., QUINN, C. E. & SELLS, I. ANN. (1980). Contamination of pathogen-free seed potato stocks by Erwinia carotovora during multiplication: results of a six year monitoring study. Potato Res. 23, 413-425.

QUINN, C. E., HARPER, P. C. & GRAHAM, D. C. (1976). Effect of fumigation of seed potato tubers with 2-aminobutane on plant growth and yield. Potato Res. 19, 147-155.

QUINN, C. E., SELLS, I. ANN & GRAHAM, D. C. (1980). Soft rot Erwinia bacteria in the atmospheric bacterial aerosol. J. Appl. Bact. 49, 175-181.

ROCA, W. M., ESPINOSA, N. O., ROCA, M. R. & BRYAN, J. E. (1978). A tissue culture method for the rapid propagation of potatoes. Amer. Potato J. 55, 691-701.

ROVIRA, A. D. (1965). Interactions between plant roots and soil micro-organisms. Annu. Rev. Microbiol. 19, 241-266.

STARR, M. P. & CHATTERJEE, A. K. (1972). The genus Erwinia: Enterobacteria pathogenic to plants and animals. Annu. Rev. Microbiol. 26, 389-426.

STARR, M. P. & WEISS, J. E. (1943). Growth of phytopathogenic bacteria in a synthetic asparagin medium. Phytopathology 33, 314-318.

STEWART, D. J. (1962). A selective diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. Nature, Lond. 195, 1023.

TUCKER, G. G. (1978). Survey of pesticide usage on potatoes stored from the 1975 Scottish crop. The Seed Potato 18, 17-22.

VRUGGINCK, H. & MAAS GEESTERANUS, H. P. (1975). Serological recognition of Erwinia carotovora var. atroseptica, the causal organism of potato blackleg. Potato Res. 18, 546-555.

WALDEE, E. L. (1945). Comparative studies on some peritrichous phytopathogenic bacteria. Iowa State Coll. J. Sci. 19, 455-484.

WHITEHEAD, T., MCINTOSH, T. P. & FINDLAY, WM. (1953). "The Potato in Health and Disease". 3rd edition, Oliver and Boyd, Edinburgh, pp. 393-402.

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Publications in chronological order

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A Selective Medium for the Isolation of Coliform Soft Rot Bacteria from Plant Tissue

DURING further studies on black leg disease of potato, difficulty was experienced in isolating the appropriate pathogens, which were identified as *Erwinia atroseptica* (van Hall) Jennison syn. *Bacterium atrosepticum* (van Hall) Burgwitz. Even using the screening methods of Noble and Marshall[1], namely, seeding plates of meat infusion agar with contaminated material from infected stems and tubers, selection of the organisms was difficult owing to the growth of many other types of bacteria. MacConkey's lactose bile-salt neutral red agar was found to be more selective, but *Pseudomonas* spp. could not now be identified by fluorescence in ultra-violet light, although a number still grew on the plates. The selective pectate gels of Rudd-Jones[2] and others were also tried, but proved to be difficult to prepare especially with limited facilities, and were often too soft to use for plating.

One of the characters of the coliform soft rot bacteria is their ability to grow and produce an acid reaction in salicin basal-salt media. A solid medium was therefore devised which would be selective and would allow this reaction to take place as an aid to identification.

The constituents are as follows:

Salicin	10·0 gm.
Sodium taurocholate	5·0 ,,
Ammonium dihydrogen phosphate	1·0 ,,
Magnesium sulphate ($MgSO_4 7H_2O$)	0·2 ,,
Potassium chloride	0·2 ,,
Bromthymol blue (water soluble)	0·05 ,,
Agar	20·0 ,,
Distilled water	1 litre

The ingredients are mixed in a flask and dissolved by steaming. The *p*H is corrected to 7·0, the medium tubed and sterilized by bringing the pressure in the autoclave to 15 lb. momentarily, then switching off. In preliminary experiments sodium pectate was substituted for salicin in the medium, but growth was unsatisfactory.

Erwinia spp. and *Aerobacter* spp. produce roughly circular, entire, finely granular, yellow to yellowish-orange colonies about 1 mm. in diameter after four days at 26° C. Colonies should be removed to meat infusion agar slopes as soon as possible because they

quickly loose viability on the medium. To differentiate between *Erwinia* spp. and *Aerobacter* spp., slices of potato tuber should be inoculated in the usual way.

Although the medium is highly selective, at times pectinase-producing *Pseudomonas* spp. have occurred on the plates. However, they form circular, entire, colourless or very pale yellow colonies, and do not grow in liquid salicin basal-salt media. On one occasion, an organism of the *Bacillus mascerans-polymyxa* group was obtained from the plates.

The medium has been used principally in black leg investigations; but isolations of soft rot coliforms from tomato, celery, etc., have been made successfully.

Studies by one of us (D. C. G.) have recently shown that while *E. atroseptica* always produces alkali in the ethyl alcohol peptone medium of Noble and Marshall[1], this reaction is not specific to the black leg organisms. Certain isolates, identified by other biological and biochemical tests as *E. carotovora* and *E. aroideae*, give the alkaline reaction also. Micro-tests, using washed suspensions in weak phthalate buffer plus 5 per cent ethyl alcohol, were carried out. These showed that acid production in the ethyl alcohol peptone was perfectly correlated with acid production in ethyl alcohol alone. A full report will be published elsewhere.

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Oct. 9.

[1] Noble, M., and Marshall, M., *Plant Path.*, **1**, 134 (1952).
[2] Rudd-Jones, D., *Nature*, **158**, 625 (1946).

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THE CONTROL OF *RHIZOCTONIA SOLANI* ON POTATO

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Routine disinfection of seed potatoes against *Rhizoctonia solani* Kühn, and other surface-borne diseases has been carried out in Scotland. Certain short dips, namely, for 15 minutes in 0·15 per cent phenyl mercury acetate solution in water, or in an aqueous solution containing 0·1 per cent each of phenol, mercuric chloride and common salt (with or without a wetting agent) and followed by a short rinse in plain water, have been used for some time. The efficacy of the treatments has, however, proved to be rather inconsistent as regards the control of *R. solani*. Therefore, studies were undertaken to determine the actual extent of control of tuber-borne infection of *R. solani* obtainable by these treatments as compared with the older dips in 0·1 per cent mercuric chloride, or 0·2 per cent mercuric chloride acidified with hydrochloric acid (Hough and Mason, 1951). Other dips were also tried in a search for quicker disinfection methods. Details of the treatments were as follows :

1. Phenyl mercury acetate (PMA). 15 minutes dip in 0·15 per cent solution of a five per cent commercial product in water.
2. Mercuric chloride. 90 minutes dip in 0·1 per cent solution in water, followed by a thorough rinse for 15 minutes in three changes of water.
3. Acidulated mercuric chloride. 5 minutes dip in 0·2 per cent solution of mercuric chloride in water plus 1·0 per cent hydrochloric acid, followed by a wash as in treatment 2.
4. Phenol-mercuric chloride. 15 minutes dip in an aqueous solution containing 0·1 per cent each of phenol, mercuric chloride and common salt, followed by a short rinse in plain water.
5. Phenol-mercuric chloride as in treatment 4, plus a wetting agent.
6. Ethoxyethylmercury chloride (EMC). 15 minutes dip in 0·5 per cent solution of a 6 per cent product in water.
7. Ethoxyethylmercury chloride plus a wetting agent. As in treatment 6.
8. Rimocidin sulphate (an antibiotic fungicide). 15 minutes dip in 0·01 per cent solution in water.
9. Hypochlorite. 30 minutes dip in 5 per cent solution of commercial product, acidified with $\frac{1}{10}$ of its volume of M sodium bisulphate, followed by a short rinse in water.
10. 2 : 4-dichloro-6-(O-chloranilino)-s-Triazine. 15 minutes dip in 0·1 per cent solution in water.

Tubers showing a number of sclerotia, which could be visually classified into negligible, thin, medium and thick types (Plate II, 2), were selected and dipped in the various solutions. This was intended to simulate commercial practice as against the studies of Guillemat and Lelièvre (1952) which were undertaken on detached sclerotia of one size only. The efficacy of the treatments was judged by observing the germination of the various categories of sclerotia on potato dextrose agar. Suitable controls were maintained. The results are shown in the following table.

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* Colombo Plan Fellow from Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture, Government of India.

EFFECT OF VARIOUS TREATMENTS ON GERMINATION OF SCLEROTIA OF R. SOLANI

	Number of Sclerotia							
	Negligible		Thin		Medium		Thick	
	+	−*	+	−	+	−	+	−
1. Phenyl mercury acetate ..	4	13	15	19	55	18	36	2
2. Mercuric chloride	0	12	1	11	5	7	4	4
3. Mercuric chloride (acidified) ..	0	12	1	10	7	4	6	1
4. Phenol-mercuric chloride ..	0	7	6	6	10	2	10	0
5. Phenol-mercuric chloride plus wetting agent	0	11	4	16	10	5	8	0
6. Ethoxyethylmercury chloride ..	0	20	1	19	0	20	7	10
7. Ethoxyethylmercury chloride plus wetting agent	0	20	0	20	1	23	2	13
8. Rimocidin sulphate	8	1	10	0	9	0	8	0
9. Hypochlorite (acidified with $NaHSO_4$)	2	10	14	5	14	3	24	0
10. 2 : 4-dichloro-6-(O-chloranilino)-s-Triazine	14	6	19	1	20	0	20	0
11. Control (no treatment).. ..	17	7	35	0	26	0	28	0

* + = germination ; − = no germination.

It was observed that all mercurial dips except PMA inhibited the germination of all negligible sclerotia ; the thin ones reacted inconsistently to PMA and phenolmercuric chloride treatments but were virtually killed by mercuric chloride and EMC dips ; the medium and thick sclerotia were, on the whole, relatively invulnerable. The inability of some of the untreated, negligible sclerotia to germinate in certain cases seems to indicate that some of them die out in the course of time owing to natural causes, such as desiccation.

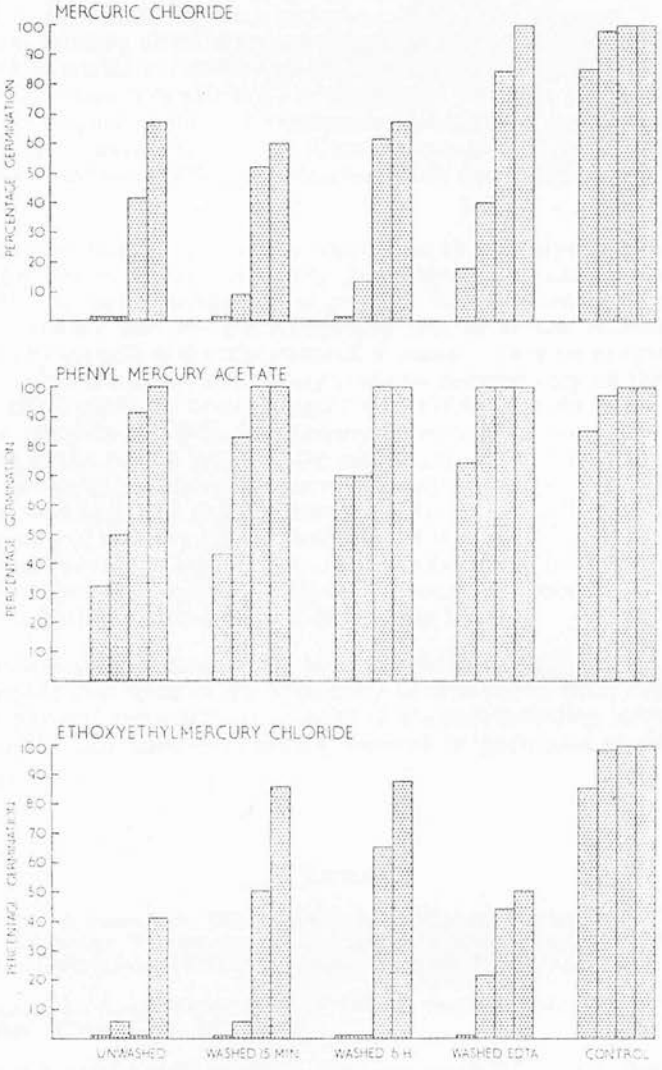
A preliminary experiment was also performed in which sets of tubers were left unwashed up to 16 days after treatment with PMA and the mercuric chloride dips, with a view to investigate the effect of any possible translocation and absorption of the chemical into the inner layers of especially thicker sclerotia in the course of time. No increase in the efficiency of the treatments was noticed.

It would appear, therefore, that until better products become available, the best chances of control of *R. solani* on seed potato tubers lie in their treatment with EMC solution plus spreader, or, failing this, with 1/1000 mercuric chloride solution for 1½ hours. If the tubers with medium and thick sclerotia are removed before dipping there will be even better chances of control. Treatments with acidulated mercuric chloride and PMA, too, would seem to be reasonably effective in such cases, but tend to be rather inconsistent owing to varying thickness of the sclerotia.

EFFECT OF WASHING AFTER TREATMENT

In the course of the work, it was noticed that if tubers treated with mercury compounds were washed in water longer than usual there was a decrease in the efficiency of the treatments. This appeared to indicate a fungistatic rather than a fungicidal action of mercury on the sclerotia. To investigate this point, separate sets of tubers after dipping treatments 1, 2, 3 and 6 were ; (*a*) left unwashed, (*b*) washed in water for 15 minutes, (*c*) washed in water for 6 hours, and (*d*) dipped in a 0·1 per cent aqueous solution of the disodium salt of ethylenediamine tetraacetic acid (EDTA) for 5 minutes, followed by a short rinse in water. This latter treatment was included because EDTA is known to

chelate with mercury, and has been reported to revive *Alternaria* spores treated with copper fungicides (Müller and Beidermann, 1952). Between ten and twenty sclerotia of each category from each treatment (Plate II, 2) were plated out on potato dextrose agar to observe germination. The results are summarized graphically by the histogram in the figure below.



Effect of washing with water or EDTA on germination of sclerotia of *R. solani* on potato tubers treated with mercurial compounds. The four columns for each treatment represent, left to right, percentage germination of negligible, thin, medium and thick sclerotia.

It will be seen that washing in water did improve germination of even negligible and thin sclerotia treated with PMA, and of thin and medium sclerotia treated

with mercuric chloride. It also considerably increased germination of medium and thick sclerotia treated with EMC.

The effect of EDTA was more pronounced in some cases. An almost 100 per cent revival of negligible and thin sclerotia was noticed with PMA treatment. So far as mercuric chloride dips were concerned, EDTA partially reversed their effect on negligible and thin sclerotia, but increase in germination was very marked with medium and thick material. With EMC however, it seemed less efficient in bringing about a revival than mere washing in water. This may be because it is unable to chelate with the mercury atom in EMC. Also, EMC is likely to be removed more readily by washing in water for 15 minutes or longer owing to its high solubility. Considerable quantities of mercury were detected by us using a microchemical test (Cunningham and Anderson, 1954) on the sclerotia treated with EDTA, whereas very little was found on those washed in water.

Bodnar and Terenyi (1932) have pointed out that absorption of mercury into the protoplasm is necessary to bring about death, but that adsorption on the cell surface is merely inhibitory to growth. The observations reported here seem to indicate that the phenyl-mercury ions or acetate molecules are not absorbed by the cells of the sclerotium of *R. solani*. They are not even adsorbed on the surface efficiently, as mercury could be detected only on thick sclerotia, and its effect could be reversed easily by EDTA. On the other hand, with mercuric chloride or EMC, the mercury appears to be both adsorbed on the cell walls of the surface layers of the sclerotium, and absorbed into the protoplasm, the latter producing an irreversible action. This latter effect is very apparent with negligible and thin sclerotia, where all the cells are better exposed to the action of mercury. With medium and thick sclerotia, however, absorption occurs only in the outer layers, which are killed, but the interior cells retain viability. Germination of such sclerotia appears to be merely inhibited by the mercury adsorbed on the dead cells of the outer layers.

The practical significance of the above results is hard to estimate. However, it is possible that some of the apparently dead sclerotia when exposed to the various physical and chemical agencies in the soil, including leaching by rain water, might have sufficient mercury removed or inactivated to allow them to germinate.

REFERENCES

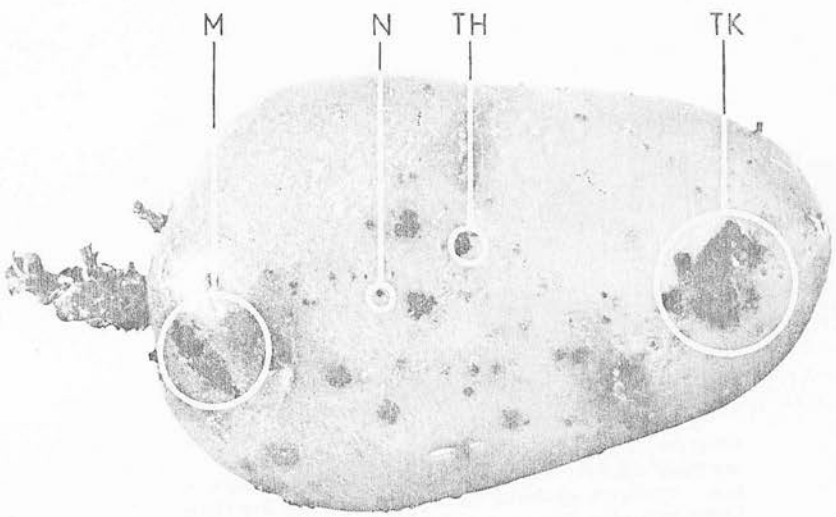
BODNAR, J. and TERENYI, A. (1932). Biochemie der Brandkrankheiten der Getreidearten : IV. Mitteilung : Wirkungsmechanismus der Quecksilbersalze auf die Weizensteinbrandsporen (*Tilletia tritici* (Bjerk.) Wint.). *Hoppe-Seyl. Z.*, **207**,78–92.

CUNNINGHAM, D. K. and ANDERSON, J. A. (1954). Rapid Detection of Mercury on Cereal Grains. *Cereal Chem.*, **31**, 513–6.

GUILLEMAT, J. and LELIEVRE, D. (1952). Comparaison de l'efficacite de Quelques Produits Chimiques sur les Sclérotes du Rhizoctone de la Pomme de Terre. *C.R., 3rd Congr. int. Phytopharmacie, Paris*, 572–81.

HOUGH, W. S. and MASON, A. F. (1951). *Spraying, Dusting and Fumigating of Plants.* Macmillan, New York and London.

MÜLLER, E. and BEIDERMANN, W. (1952). Der Einfluss von Cu^{+2}-Ionen auf den Keimungsablauf von Alternaria tenuis. *Phytopath. Z.*, **19**, 343–50.



2. Potato tuber showing negligible (N), thin (TH), medium (M) and thick (TK) sclerotia of *Rhizoctonia solani*. $\frac{3}{4}$ natural size.

PLATE II

LONDON
HER MAJESTY'S STATIONERY OFFICE
1958

(72272) Wt. 58925/23 K.2 2/58 Hw.

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Occurrence of Soft Rot Bacteria in Scottish Soils

A METHOD of isolating soft rot bacteria from soil using immature potato tubers as a form of enrichment medium was described by Kerr[1]. He found that of twenty Scottish soils examined all contained Gram-negative bacteria capable of rotting potato tuber slices.

When the term 'soft rot bacteria' is employed most workers generally assume that this implies 'coliform soft rot bacteria', that is, *Erwinia carotovora* and related species. With this assumption is associated the prevalent view that soft rot coliforms are commonly present in soils, and can be isolated readily from them. On this basis it is usually considered that the organisms isolated by Kerr were *Erwinia* spp., although he did not identify the genus to which his isolates belonged.

A study of the soft rot bacteria in Scottish soils was undertaken in connexion with investigations on black leg disease of potato, caused by *E. atroseptica*. During the years 1956 and 1957, seventeen soil samples, taken from different localities, and including peaty, shale, sandy and clay types, all under current cropping, were tested for the presence of soft rot bacteria. Kerr's technique was used, and soil suspensions were plated out directly on the salicin agar described by Noble and Graham[2], and on the pectate gel mentioned by Dowson[3]. A number of isolates were obtained from each soil sample by all three methods, which were capable of rotting potato tuber slices at 26° C. Most of these were Gram-negative bacteria, and further examination showed them to be members of the genus *Pseudomonas*. Many of these pseudomonads do not produce a fluorescent pigment on meat infusion agar, but will do so in a medium containing inorganic salts plus an organic acid such as gluconic acid, treated with 8-hydroxyquinoline to reduce the heavy metal content, after the method devised by Paton[4]. Such organisms are therefore easily confused with coliforms unless adequate tests are applied.

In other experiments, heavy suspensions of a mixture of two strains of *E. atroseptica* were poured on to 500 gm. of various soils (sandy, clay, shale, peat and unsterilized John Innes compost) contained in Kilner jars with loose-fitting lids. The jars were buried up to the neck in soil in the open during

November 1955 and 1956, while others were kept at room temperature. Using Kerr's method and the salicin agar technique, isolations from all jars, in May 1956 and 1957, yielded only soft rot organisms of the genus *Pseudomonas*, none of which could be specifically identified. Similar pseudomonads have been obtained from naturally rotting tissues of a number of plants, including potato, carrot, leek, celery and cauliflower.

Some Gram-positive organisms were associated with rotting when Kerr's technique was used for isolation, and all were members of the genus *Bacillus*. *B. polymyxa* (Voges–Proskauer positive) was fairly common, but *B. macerans* (Voges–Proskauer-negative) was obtained on two occasions only.

It is concluded that in enrichment cultures from Scottish soils the dominant soft rot organisms are pseudomonads, and not coliform bacteria.

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[1] Kerr, A., *Nature*, **172**, 1155 (1953).
[2] Noble, M., and Graham, D. C., *Nature*, **178**, 1479 (1956).
[3] Dowson, W. J., *Nature*, **179**, 682 (1957).
[4] Paton, A. M., Ph.D. thesis, University of Edinburgh (1956).

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COMMONWEALTH PHYTOPATHOLOGICAL NEWS

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MAIN ITEMS

THE STATUS OF ERWINIA LATHYRI AND RELATED BACTERIA

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Erwinia lathyri (Manns & Taubenhaus) Holland may be formally described as a Gram negative, peritrichously flagellated bacillus belonging to the Enterobacteriaceae, having as one of its most distinctive characters the ability to produce a yellowish pigment on many media. On numerous occasions in the past, E. lathyri has been credited with causing various plant diseases. The organism was first recorded by Manns and Taubenhaus in 1913, who believed it to be the causal agent of streak disease of sweet peas. Later, it was considered the cause of streak disease of tomato and chocolate spot of broad bean. The first two are now known to be virus diseases and chocolate spot is caused by Botrytis spp.

Most workers now agree that the bacterium is a saprophyte, and that its natural habitat is most probably the soil, on plants and plant debris. As a result, it is frequently found amongst the organisms isolated from lesions induced by true parasites on many kinds of plants. I have obtained it from black leg lesions on potato stems, soft rots of potato tubers, tomato, celery and cauliflower, from halo blight lesions on French bean, and from healthy leaves of Narcissus. It has also appeared on salicin-bile salt agar plates inoculated directly with soil suspensions, but not all strains grow on this medium.

Although now discredited as a pathogen, E. lathyri occurs so commonly it is worth describing its biochemical characteristics some of which I have recently recorded. It has a coliform type of metabolism, producing acid rapidly from a large number of sugars, sugar alcohols, and glycosides, but without formation of gas. All strains liquify gelatin slowly, do not liquify pectate gel, and produce small amounts of 2-ketogluconate from gluconate. Most strains give a positive test for nitrate reduction and form acid in ethyl alcohol agar, while a few produce indole. The methyl red test is usually negative and the Voges-Proskauer test positive after five days' growth in ordinary glucose-phosphate-peptone medium,

but some strains give reverse reactions.

When searching for a bacterial pathogen, especially where non-selective media are used for isolation, E. lathyri is quite likely to be found. Furthermore, although with experience colonies of E. lathyri can be distinguished from those of Xanthomonas it is usually necessary to carry out biochemical tests to confirm the identity of the organisms. For example, the writer was examining seakale root showing characteristic symptoms of infection with X. campestris, and yellow colonies appeared in almost pure culture on the isolation plates. However, investigation showed that the organism formed acid in salicin-basal salt media, and did not produce the honey-coloured slimy growth on sterilized potato characteristic of Xanthomonas. Further tests confirmed it to be E. lathyri - probably the true pathogen had already died out in the seakale before isolation was attempted. When dealing with soft rot diseases it is always advisable to check the pectolytic capacity of these yellow organisms, since chromogenic strains of Pectobacterium carotovorum occur occasionally. E. flavida (Fawcett) Magrou may be one of these.

It is noteworthy that E. lathyri is no longer described in Bergey's Manual of Determinative Bacteriology (7th edition, 1957) because of its doubtful taxonomic position. Any worker using the manual will not, therefore, be able to identify this organism although Skerman's artificial key will lead him to "probably Erwinia".

Other yellow pigmented species

A number of other pigmented Erwinia species are reported in the literature as causing plant diseases. These organisms have been given specific status depending on the plant from which they were originally isolated, without any previous comparative studies, and include E. ananas Serrano; E. cassavae (Hansford) Burkholder; E. citrimaculans (Doidge) Magrou; E. mangiferae (Doidge) Bergey et al.; E. milletiae (Kawakami & Yoshida) Magrou; and E. vitivora (Baccarini) Du Plessis. Their pathogenic properties are somewhat doubtful. For instance, Serrano claimed he could induce typical rotting of pineapple fruit with cultures of E. ananas. However, in practice it is possible to produce a variety of symptoms merely by "inoculating" fruit with a sterile needle. This is owing to the presence of organisms in the nectary ducts, blossom cups and even in the glands of the fruitlet. The process of inoculation releases the bacteria from the tissues in which they are normally confined and introduces them into the fleshy part of the fruit where they can grow. It is therefore impossible to be sure whether the symptoms develop from organisms intentionally introduced or from those present on the tissues by chance.

Culturally and biochemically all these Erwinia species seem indistinguishable from strains of E. lathyri, while the pigments appear to be of the same nature. Recently I have compared the pigments of a number of Hawaiian strains of E. ananas with those of E. lathyri. They are xanthophylls, and can be extracted readily from moist cells by refluxing with methanol, but it is not possible to dissolve them out directly with other carotenoid solvents such as diethyl ether, chloroform, petroleum ether or carbon disulphide. The absorption spectra of the pigments from E. ananas and E. lathyri are very similar if not identical, although the colouring matter from a yellow strain of Pectobacterium carotovorum is quite distinct. The nature of the pigments strengthens my view that E. ananas is merely a strain of E. lathyri, as are probably all the other species. In the circumstances, it would be worthwhile re-examining the various diseases with this in mind. Possibly the true pathogen has not been detected, as was certainly the case with cassava leaf spot. This is now known to be caused by X. cassava Wiehe & Dowson, and not E. cassavae.

Other organisms which may belong to E. lathyri are the yellow chromogenic coliforms which have long been known to dairy bacteriologists and have been identified as Aerobacter aerogenes or A. cloaceae. Since these workers are dealing with material which may be contaminated directly or indirectly with soil and plant bacteria it is not surprising that such organisms are found from time to time. I have examined one such isolate, received as A. cloaceae. Apart from its capacity to produce gas from sugars it is biochemically like E. lathyri, and its pigment is a xanthophyll with properties similar to that of E. lathyri. Obviously it would be advantageous if there was more opportunity for exchanges of views between plant

bacteriologists and those working in medical, dairy and other fields. At least it would help to clear up some of the confusion that exists regarding the nomenclature and taxonomy of the plant bacteria.

DETERIORATION OF GLASSWARE IN THE TROPICS

by R.N. Hilton
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Although it is generally known that cameras and microscopes must be kept dry, nothing appears to have been written on the nature of the deterioration of glass apparatus in the tropics and how to prevent it until Mr. Dade's article on this topic in the April issue of C.P. NEWS. This must have been as interesting to other workers in the tropics as it was to us in the Pathological Division of the Rubber Research Institute of Malaya. It may be of interest to others to know what routine precautions we have been taking over the past ten years, successfully, but often without knowing the scientific basis for them.

The whitening in storage of microscope slides, cover glasses, measuring cylinders, and soda-glass apparatus long puzzled us and was put down to "devitrification". In some books it is stated that the white deposit can be removed by soaking the glassware in a 5 per cent. aqueous solution of sodium metasilicate. We have found only hydrofluoric acid to work. As much of the price of glassware lies in carriage we now buy Pyrex glassware which does not suffer from this defect under our laboratory conditions. The custom some makers have of packing microscope slides for tropical use in acid alcohol is quite unnecessary as dry slides are easier to pack, and any laboratory can provide acid alcohol to store them in on arrival. We always put slides and cover glasses, whether in store or on the bench, straight into 70 per cent. alcohol to which a few drops of hydrochloric acid have been added.

Many people have spoilt their cameras or binoculars by sealing them away hermatically. The best advice to those with no special facilities is to take advantage of periods of low humidity, and open their optical instruments for a few hours in the middle of the day. We have kept microscopes on a glass plate on the bench, under bell jars with ground glass edges. A jar of calcium chloride is put in as a desiccant, this having to be changed less frequently than silica gel. Each microscope has an ordinary desiccator standing by it containing accessories, over calcium chloride. We have not experienced the trouble that is supposed to result from over-drying, endangering the balsam mounts of the lenses, but some recommend quick lime as a milder desiccant.

The advent of the air-conditioned laboratory and store-room has solved these problems, but it will be a pity if the experience gained in running laboratories at relative humidities up to 100 per cent. and temperatures of 70° - 90°F. is lost. Even though the larger research establishments are likely to be air-conditioned, small laboratories and schools will benefit from following the few simple precautions outlined.

VISIT OF THE FRENCH MYCOLOGICAL SOCIETY TO THE C.M.I.

On the morning of Sunday September 7th about thirty members of the French Mycological Society who were holding a joint foray with the British Mycological Society at Reading visited the Commonwealth Mycological Institute. The Assistant Director, Major Dade, and members of the scientific and technical staff attended to explain the work and aims of the C.M.I. The visiting mycologists spent an hour and a half in the herbarium and culture collection and were greatly interested in everything that was shown them. Special interest was shown in the way specimens, slides and dried cultures illustrating the life histories of fungi are all filed away together in the herbarium folders. Slide culture techniques and the drying down of cultures also attracted considerable attention. Specimen copies of the REVIEW OF APPLIED MYCOLOGY and other publications were on show; taxonomists amongst the visitors were particularly interested in the MYCOLOGICAL PAPERS which some of them had not seen before.

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THE COLIFORM BACTERIA ASSOCIATED WITH POTATO BLACK-LEG AND OTHER SOFT ROTS

I. THEIR PATHOGENICITY IN RELATION TO TEMPERATURE

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(With Plate 2)

Twelve glasshouse trials using twenty-five isolates of coliform soft-rot bacteria obtained from various countries showed that all the isolates produced typical black-leg of potato at temperatures of 76° F. (24·5° C.) or over, while some of them could also produce the disease at temperatures below 66° F. (19° C.).

Thus the isolates fall into two main groups of which the high-temperature group originates mostly from tropical or subtropical countries or from plants grown in heated glasshouses, whereas the low-temperature group is indigenous in the north temperate regions. The high-temperature group comprises *Pectobacterium carotovorum* (*Erwinia carotovora*), *P. carotovorum* var. *chrysanthemi* (*E. chrysanthemi*) and *P. carotovorum* var. *aroideae* (*E. aroideae*). The low-temperature group consists of one variety, *P. carotovorum* var. *atrosepticum* (*E. atroseptica*).

This temperature relation provides a possible means of discovering the origin of the black-leg pathogen in crops grown from Scottish seed in certain tropical or subtropical countries.

As a result of this investigation it is considered that all soft-rot coliform bacteria are varieties of a single species, *P. carotovorum* (Jones) Waldee.

INTRODUCTION

The problem of interrelations within the complex group of organisms known as the 'soft-rot coliform bacteria' has existed for more than half a century. Ever since Jones (1901) described *Bacillus carotovorus* as the cause of soft rot of carrot, van Hall (1902) *B. atrosepticus* as the cause of potato black-leg and Townsend (1904) *B. aroideae* as the cause of soft rot of arum lily, workers have disputed whether these three should be designated as different species, or variants of a single species. In 1920, Erwin Smith stated that *B. phytophthorus* (the name given to *B. atrosepticus* by Appel (1902)) was 'not sufficiently distinguished from *B. carotovorum* Jones'. Notwithstanding the protracted study of these organisms, the position still remains essentially the same.

The nomenclature of the soft-rot bacteria has become confused because several generic names have been proposed for them. American workers adopted the genus *Erwinia* Winslow *et al.*, and when the genus *Bacillus* Cohn was made invalid for rod-shaped non-sporing organisms, British workers placed the plant pathogenic

coliforms in the genus *Bacterium* Ehrenberg. However, *Bacterium* was rejected by international agreement in 1953, and this decision forced British bacteriologists to use a new name. Some immediately accepted *Erwinia*, but Dowson suggested the adoption of the *genus* Pectobacterium (Waldee, 1945) for the soft-rot organisms, leaving *Erwinia* for the coliforms causing dry necroses, galls or wilts. Although this suggestion has not yet been generally accepted, we propose to use the name *Pectobacterium* for the organisms discussed in this and the following paper.

The basis for separating the soft-rot coliform bacteria into species, variants and *forma speciales* has always been controversial. It has rested largely on characteristics of fermentative ability and pathogenicity, while little attention has been paid to serology or the newer tests now widely employed by medical bacteriologists, such as growth in cyanide media and production of amino-acid decarboxylases. Much of the work on pathogenicity has been unsatisfactory, most of the tests having been made by inoculating soft, fleshy plant organs such as potato tubers, cucumber fruits and onions. Amongst growing plants the potato has been most often used because of its commercial significance and the fact that potato black-leg is probably the most important bacterial soft-rot disease. Some of the earlier investigators found that certain isolates when inoculated into potato stems readily produced black-leg disease, while other isolates did not; and it was noted that those producing black-leg had almost invariably been isolated from potato, while the remainder usually came from other hosts. Coupled with this were certain biochemical differences; and some workers considered that the etiological agent of black-leg disease should be separated from the other soft-rot coliforms and called *Erwinia atroseptica* (van Hall) Jennison (*P. carotovorum* (Jones) Waldee var. *atrosepticum* Dowson). The most recent exponents of this view are Burkholder & Smith (1949), who maintained there were two distinct species, *E. atroseptica* and *E. carotovora*. At that time they did not recognize the existence of *E. aroideae*, generally regarded as an organism which produces acid but no gas from carbohydrates. Later, however, Holdeman & Burkholder (1956) claimed to have isolated 'true' anaerogenic *E. aroideae* from tobacco.

In recent years other investigators, notably Jones (1950) and Hellmers & Dowson (1953), claimed it was possible to produce black-leg of potato with all soft-rot coliform isolates. Hellmers & Dowson considered that inconsistency in the results of pathogenicity tests could be explained through their discovery that the bacteria must be introduced directly into the vascular bundles to obtain infection. Using this method, they were able to cause black-leg with an American isolate which was said by Burkholder & Smith to be non-pathogenic towards potato stems.

Because published work proved so contradictory, experiments on potato black-leg were started at Scientific Services in 1951. Black-leg is important in connexion with the Certification Scheme and the seed potato export trade, and the work reported here is part of a re-examination of all aspects of the disease being carried out at Edinburgh.

Pathogenicity tests on potato plants

Early experiments at Edinburgh tended to confirm the view of Burkholder & Smith that at least two species were involved, and some isolates were found to be unable to produce black-leg even when introduced directly into the vascular tissue of the stem (Noble & Marshall, 1952). In 1955–6 work was continued by D. C. Graham using a much greater range of isolates, and employing Hellmers & Dowson's technique. Again the results were inconsistent, so joint work was arranged to discover the reasons for these discrepancies. We carried out two tests together at Cambridge in April and May 1957 and then a series of ten further experiments independently in Cambridge and Edinburgh throughout 1957 and 1958.

Materials and methods

During the twelve trials, twenty-five isolates were employed, but not all were used in every test. The source and identity of each isolate is given in Table 1. Cultures of particular interest include Holdeman & Burkholder's 'true *aroideae*' (strain H2); the organisms causing slow wilt of carnation (Lelliott, 1956) (strain 402), bacterial blight of chrysanthemum (Burkholder, McFadden & Dimock, 1953) (strain 399), and bacterial disease of guayule (Starr, 1947) (strain 398); and also the organism recently found to be the cause of soft rot of pineapple in Malaya (Johnson, 1957) (strain 1172). Strain 377 is also of interest, as we consider it to be identical with the organism described by Sabet (1954) as *E. carotovora* f.sp. *zeae*, the cause of bacterial rot of maize in Egypt. Strain EP3 is the organism causing leaf rot of *Philodendron* described by Miller (1956) from Florida. Cultures were grown on meat infusion agar slopes for 24 hr. at 27° C. and the growth emulsified in 5 ml. water for inoculation.

Potato plants were grown in 6 in. pots of sterile soil in cool glasshouses, using the variety Sharpes Express at Cambridge and Majestic at Edinburgh. Both varieties are known to be susceptible to black-leg from field observations.

Inoculation wounds were made with sterile safety-razor or scalpel blades as described by Hellmers & Dowson (1953), by cutting across the wings on the stems, going deep enough to sever one of the main vascular bundles. The bacterial suspension was inserted into the wounds with sterile glass pipettes, and the wounds bound with 1 in. wide self-adhesive tape. Controls were inoculated with sterile water. Each stem was usually inoculated in two places, one at the base about 1 in. above soil level, the other 3–4 in. below the growing point. In each experiment all the stems of between four and ten plants were inoculated with each isolate, with three plants as checks. At Cambridge the plants were covered with large bell jars and kept in the glasshouse, but at Edinburgh, plants were maintained in small separately heated and ventilated cubicles in the glasshouse. Humidity was kept near 100% by frequent damping down of the walls and floor. The maximum and minimum air temperatures were recorded daily so that the average temperatures could be calculated for the duration of the tests, which usually lasted about 7 days.

TABLE 1. *Isolates used in black-leg inoculations*

Ref. no.	Plant	Source	Date of isolation	Name of organism as received	Name of organism considered to be correct
SR2/1	Potato stem	S. Rhodesia	1955	—	*P. carotovorum* var. *atrosepticum*
HT/1	Potato stem	Scotland	1955	—	*P. carotovorum* var. *atrosepticum*
QB4	Potato stem	Scotland	1955	—	*P. carotovorum* var. *atrosepticum*
123	Potato	U.S.A.	?	*E. aroideae*	*P. carotovorum* var. *aroideae*
14B	Potato tuber	England	1954	*E. carotovora*	*P. carotovorum*
340	Avocado pear	Israel	1954	*E. carotovora*	*P. carotovorum*
312	Potato tuber	Denmark	1952	*E. carotovora*	*P. carotovorum*
377	Maize	S. Rhodesia	1956	*E. carotovora*	*P. carotovorum*
H2	Tobacco	U.S.A.	1955	*E. aroideae*	*P. carotovorum* var. *aroideae*
EP3	Philodendron	U.S.A.	1955	*E. chrysanthemi* var. *philodendri*	*P. carotovorum* var. *chrysanthemi*
370	Calla Lily	Denmark	1955	*E. aroideae*	*P. carotovorum* var. *aroideae*
A81V	Avocado pear	Israel	1954	*E. aroideae*	*P. carotovorum* var. *aroideae*
398	Parthenium	U.S.A.	1946	*E. carotovora* f.sp. *parthenii*	*P. carotovorum* var. *chrysanthemi*
399	Chrysanthemum	U.S.A.	1955	*E. chrysanthemi*	*P. carotovorum* var. *chrysanthemi*
402	Carnation	Denmark	1955	*E. carotovora* f.sp. *dianthicola*	*P. carotovorum* var. *chrysanthemi*
39	Potato stem	Scotland	1957	—	*P. carotovorum* var. *atrosepticum*
H25	Potato tuber	Denmark	1952	*E. atroseptica*	*P. carotovorum*
H35	Potato tuber	Denmark	1952	*E. carotovora*	*P. carotovorum*
H904	Iris	U.S.A.	1946	*E. carotovora*	*P. carotovorum*
C1	Potato	Israel	1956	*E. atroseptica*	*P. carotovorum* var. *atrosepticum*
C5	Potato	Israel	1956	*E. atroseptica*	*P. carotovorum* var. *atrosepticum*
119V	Tomato fruit	Israel	1958	*E. aroideae*	*P. carotovorum* var. *aroideae*
B1	Potato stem	Scotland	1957	—	*P. carotovorum* var. *atrosepticum*
74V	Maize	Israel	1957	*E. aroideae*	*P. carotovorum* var. *aroideae*
1172	Pineapple	Malaya	1956	*E. carotovora*	*P. carotovorum*

Results of inoculations

In the first trial at Cambridge during the period 29 April 1957 to 5 May 1957, strains H25, H35, H904, 123, SR2/1, H2, EP3, and 377 were used and of these only SR2/1 and H904 gave a black-leg reaction; the former caused rapid rotting, wilting and collapse, but the latter was only weakly pathogenic with slow spread of the rot. Most of the strains which failed to produce the typical disease gave only small localized lesions at the site of inoculation. These were the results which Graham had predicted from previous experience of the isolates. We quickly realized that the plants had been kept in a cold glasshouse and we considered that temperature of incubation might have a more significant effect than was generally realized. Accordingly, a second experiment was carried out between 3 May 1957 and 9 May 1957 using similar plants, but these were transferred to a heated glasshouse after inoculation. The effect was most striking, all strains producing severe black-leg symptoms within 4 days. It thus appeared that temperature was the controlling factor in disease production, and the further ten tests, using an increased number of strains, amply confirmed our first impressions. Unfortunately, neither

TABLE 2. *Summary of results of temperature infection experiments*

	Temperature ranges					
	Lower		Higher			
Strain	60–65° F.	66–70° F.	71–75° F.	76–80° F.	81–85° F.	86–90° F.
SR2/1*	+ + +	+ + + +	+	+ + +	+ +	+ +
HT/1*	+ +	+ +	+	+	+ +	+
QB4*	+ +	+ +	+	+	+ +	+
123	− −	− + +	+	+	+	+
14B	− −	− −	−	.	+	+
340	−	−	−	+	+	+
312	−	+	−	.	.	+
377	− −	− −	−	− +	+	+ +
H2	−	− − −	−	− +	+ +	+
EP3	−	+ −	+	+ +	+	+
370	−	−	+	+	+	+
A81V	−	− + +	.	+	+	+
398	− −	− +	+	.	+	+
399	− −	+ +	+	.	+	+
402	− −	− −	+	.	+	+
39*	+ +	+ +	.	+	+	+
H25	.	− +	+	+	.	.
H35	.	− +	.	+	.	.
H904	.	+	.	+	.	.
C1*	+	+	.	+	+	+
C5*	+	+	.	+	+	.
119V	−	−	.	+	+	+
B1*	+	+	.	+	.	+
74V	−	−	.	+	.	+
1172	−	−	.	+ +	+	+

Each positive or negative sign represents the result of a test carried out within a given average temperature range. The asterisk marks those strains that infect at the lowest temperature, i.e. below 66° F. These correspond to *Pectobacterium carotovorum* var. *atrosepticum*.

+ve = black-leg production; −ve = no reaction; . = reaction not recorded.

of us had controlled-environment equipment at our disposal, but by adjusting the heating and shading, and the use of extractor fans in the glasshouse, we obtained considerable temperature ranges.

Table 2 summarizes the results of all experiments, but only the average temperatures throughout the duration of each experiment are given and these are grouped within arbitrary temperature ranges for ease of presentation. The reactions of the variety Majestic inoculated with a strain capable of causing black-leg at a high temperature only (strain 377) and kept at high and low temperatures are illustrated in Pl. 2.

DISCUSSION

The discovery of this temperature-pathogenicity relation is important in regard to the controversy as to whether or not all soft-rot coliform bacteria are capable of causing black-leg of potato. Examination of the sources of isolates shows that most of the group of organisms causing black-leg only at the higher temperatures correspond to the *E. carotovora*, *E. chrysanthemi* and *E. aroideae* of American workers (our *P. carotovorum*, *P. carotovorum* var. *chrysanthemi* and *P. carotovorum* var. *aroideae*, respectively). These usually originated in the tropics or subtropics, or were found associated with such plants as calla lilies, which are grown in heated glasshouses in temperate climates. Such isolates, pathogenic only at higher temperatures, are obviously strains evolved under warmer conditions than are normally experienced in Britain. Similarly the group of isolates inducing black-leg at the low temperatures correspond to the American *E. atroseptica* (our *P. carotovorum* var. *atrosepticum*), and it is this organism which is the causal agent in the cool climate of Scotland. Since 1955, more than 200 isolations have been made from sixteen different black-leg-infected potato varieties, and the pathogenicity of many of the isolates has been checked. All belong to the low-temperature group, and as far as can be ascertained the Scottish black-leg organism is a perfectly homogeneous variant.

Furthermore, this temperature relation provides a possible means of discovering the origin of black-leg infection in crops grown from imported Scottish seed in certain tropical and subtropical countries. For instance, the organisms obtained from Israel and Southern Rhodesia, isolated from potatoes grown from Scottish seed, were found to belong to the low-temperature group; a result which additionally supports the view that the black-leg pathogen is carried with the tuber to the importing country, and that the disease there need not be caused by indigenous strains of soft-rot coliforms.

Finally, we consider that the results lend support to the view that all soft-rot coliform bacteria are variants of a single species, *P. carotovorum*.

We would like to thank Prof. W. H. Burkholder, U.S.A.; Dr G. R. Bates, Southern Rhodesia; Dr E. Hellmers, Denmark; Mr A. Johnson, Thailand; Dr Jean F. Malcolmson, Scotland and Dr Z. Volcani, Israel, for providing cultures of bacteria used in these experiments.

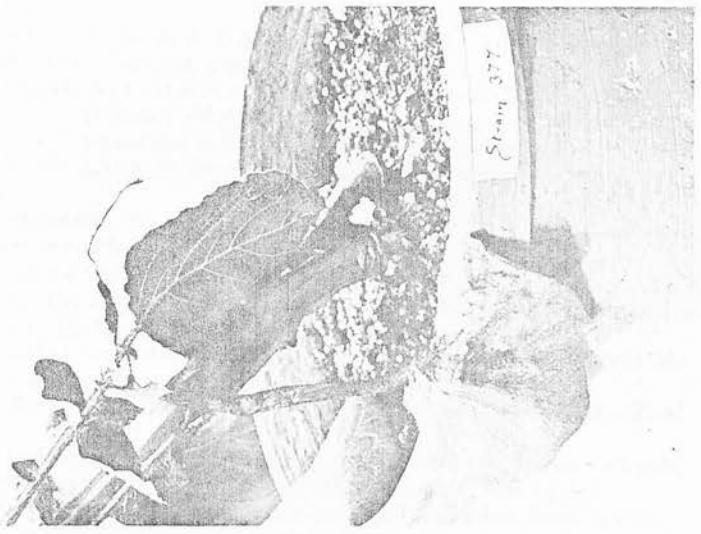

Fig. 2

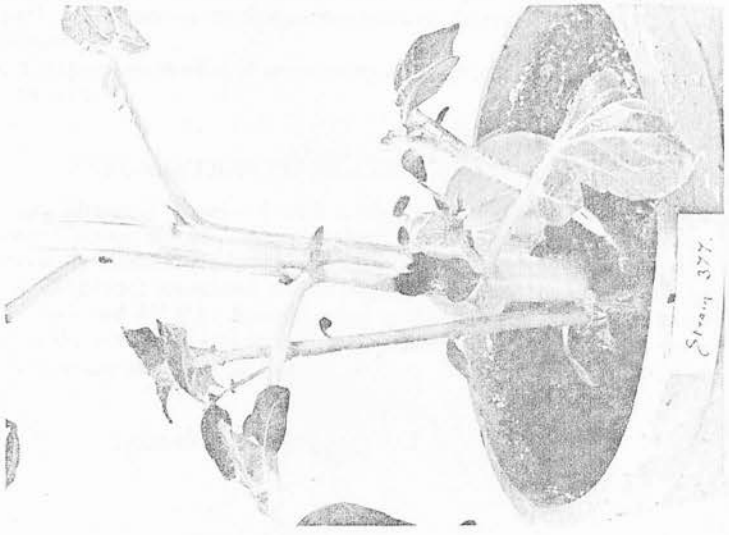

Fig. 1

GRAHAM AND DOWSON—*Coliform bacteria associated with potato black-leg. I*

(*Facing p.* 56)

REFERENCES

APPEL, O. (1902). Zur Kenntnis der Bacterienfaule der Kartoffeln. *Ber. dtsch. bot. Ges.* **20**, 128.

BURKHOLDER, W. H., MCFADDEN, L. A. & DIMOCK, A. W. (1953). A bacterial blight of chrysanthemums. *Phytopathology*, **43**, 522.

BURKHOLDER, W. H. & SMITH, W. L. (1949). *Erwinia atroseptica* (van Hall) Jennison and *Erwinia carotovora* (Jones) Holland. *Phytopathology*, **39**, 887.

DOWSON, W. J. (1957). *Plant diseases due to bacteria.* Cambridge University Press.

HELLMERS, E. & DOWSON, W. J. (1953). Further investigations of potato black-leg. *Acta agric. Scand.* **3**, 103.

HOLDEMAN, Q. L. & BURKHOLDER, W. H. (1956). The identity of barn rots of flue-cured tobacco in South Carolina. *Phytopathology*, **46**, 69.

JOHNSON, A. (1957). Pineapple fruit collapse. *Malayan agric. J.* **40**, 253.

JONES, D. RUDD (1950). On the nomenclature and identity of the coliform soft-rot bacteria. *Trans. Brit. mycol. Soc.* **33**, 73.

JONES, L. R. (1901). *Bacillus carotovorus* n.sp., die Ursache einer weichen Fäulnis der Mohre. *Zbl. Bakt.* **2**, Abt. 7, 12 and 61.

LELLIOTT, R. A. (1956). Slow wilt of carnation caused by a species of *Erwinia*. *Plant Path.* **5**, 19.

MILLER, H. N. (1956). In *Annual Report of the Agricultural Experiment Stations, Florida, for the year ending* 30 *June* 1956. (Abstr. in *Rev. appl. Mycol.* **36**, 808, 1957.)

NOBLE, M. & MARSHALL, N. (1952). A note on black-leg of potato. *Plant Path.* **1**, 134.

SABET, K. A. (1954). A new bacterial disease of maize in Egypt. *Emp. J. exp. Agric.* **22**, 66.

SMITH, E. F. (1914). *Bacteria in Relation to Plant Diseases*, **3**, 282.

STARR, M. P. (1947). The causal agent of bacterial stem and root disease of guayule. *Phytopathology*, **37**, 291.

TOWNSEND, C. O. (1904). A soft-rot of the calla lily. *Bull. U.S. Bur. Plant Ind.* no. 60.

VAN HALL, C. J. J. (1902). Bijdragen tot de Kennis der Bakterieele Plantenziekten. *Inaug. Diss., Univ. Amsterdam.*

WALDEE, E. L. (1945). Comparative studies of some peritrichous phytopathogenic bacteria. *Iowa Coll. J. Sci.* **19**, 435.

EXPLANATION OF PLATE 2

Fig. 1. Potato plant (var. Majestic) inoculated with a high-temperature strain, and kept at a low temperature (average 62° F.). Photographed 7 days after inoculation. Note the localized lesion on the stem; there is no spread of the bacteria from this region.

Fig. 2. Potato plant (var. Majestic) inoculated with a high-temperature strain and kept at a high temperature (average 88° F.). Photographed 4 days after inoculation. Note the typical wilting of the plant and blackening of the stem base; the organisms have also spread down the petiole and into the leaf.

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THE COLIFORM BACTERIA ASSOCIATED WITH POTATO BLACK-LEG AND OTHER SOFT ROTS

II. BIOCHEMICAL CHARACTERISTICS OF LOW- AND HIGH-TEMPERATURE STRAINS

By D. C. GRAHAM and W. J. DOWSON

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The biochemical characteristics of twenty-five isolates of soft-rot coliform bacteria from different countries are recorded. Because of the variability of the reactions, strains capable of producing potato black-leg at both low and high temperatures, or at higher temperatures only, cannot be distinguished from one another with certainty by biochemical tests alone. They do, however, give an indication of potential pathogenic reactions. Thus the low-temperature strains correspond with *Pectobacterium carotovorum* var. *atrosepticum* and are distinguished from the high-temperature isolates by their gas production, rapid fermentation of lactose and maltose, methyl red and Voges–Proskauer reactions and failure to give a positive indole test.

Because of their delayed lactose fermentation the chrysanthemum blight pathogen, the carnation slow wilt organism, the guayule bacterium and the organism causing leaf rot of *Philodendron* are classified as a new variety of *P. carotovorum* (Jones) Waldee.

The diagnosis of the coliform soft-rot bacteria should be based on qualitative and quantitative biochemical reactions rather than on host range.

INTRODUCTION

In the preceding paper we pointed out that all isolates of soft-rot coliform bacteria were capable of causing black-leg in the glasshouse if the inoculated plants were incubated at appropriate temperatures, and that strains could be conveniently divided into low- and high-temperature groups. Those producing black-leg below an average temperature of 66° F. (19° C.) were considered to correspond to *Pectobacterium carotovorum* var. *atrosepticum*.

Biochemical tests were made on the isolates concurrently with the pathogenicity experiments. This was done, first, to confirm the identity of the organisms as soft-rot coliform bacteria; and secondly, to find whether any biochemical reaction or group of reactions would distinguish between the low- and high-temperature strains. For details of the organisms employed in the biochemical tests, the reader is referred to Table 1 in Graham & Dowson (1960).

Biochemical tests

Methods

Fermentation tests. Salicin, sucrose, glucose, lactose and maltose were incorporated at 1% concentration in an inorganic (basal) medium, using ammonium phosphate as a nitrogen source, together with bromothymol blue as an indicator, at pH 7·2. Final readings were taken after incubation for 21 days at 26° C.

Liquefaction of gelatin. Liquefaction was observed in nutrient gelatin stab cultures incubated for 30 days at 22° C.

Reduction of nitrate. Nitrate reduction was determined by growing the organism in a medium containing 1% peptone (Evans) and 0·1% potassium nitrate for 48 hr. at 26° C. and testing for the presence of nitrite with the dimethyl-1-naphthylamine sulphanilic acid reagent.

Liquefaction of pectate gel. Stab cultures were made in Wieringa's pectate gel medium; details of preparation are given by Dowson (1957). Cultures were incubated at 26° C., and readings taken after 14 days.

Methyl red and Voges–Proskauer tests. Organisms were grown in ordinary glucose–phosphate–peptone (Evans) medium for 5 days at 26° C. The culture fluid was then divided into two, one portion being tested with methyl red indicator, the other for the presence of acetylmethylcarbinol by the caustic potash–creatine method.

Gluconate test. The medium employed consisted of potassium gluconate 40·0 g.; peptone (Evans) 2·5 g.; K_2HPO_4 1 g.; distilled water 1000 ml.; adjusted to pH 7·0; sterilized at 10 lb./sq.in. for 10 min. After incubation of the culture for 48 hr. at 26° C., an equal volume of Benedict's qualitative reagent for reducing sugars was added and the tubes placed in boiling water for 10 min. In this test a positive reaction is indicated by a heavy yellowish-brown precipitate of cuprous oxide.

Ethyl alcohol agar test. Cultures were grown on alcohol agar prepared according to the method of Massey (1924). They were incubated at 26° C., and final readings were taken after 21 days.

Indole production. Organisms were grown in 1% Bacto–Tryptone broth for 48 hr.; the presence of indole was detected with Kovacs's *p*-dimethylaminobenzaldehyde reagent.

Hydrogen sulphide. A similar medium to that used for the indole test was employed. H_2S was detected by lead acetate papers suspended above the medium. Cultures were incubated for 21 days at 26° C. before final readings were taken.

Results

Table 1 summarizes the results of the tests made on the twenty-five isolates of soft-rot coliform bacteria. None of the tests noted here, except the gluconate reaction, is new, since it had been found that most of the newer tests, such as the production of various amino-acid decarboxylases and phenylalanine deaminase, so commonly used in studies on other coliform bacteria (cf. Cowan, 1956), would not distinguish between the various strains of soft-rot coliforms (Graham, unpublished). The gluconate test, which depends on the oxidation of gluconate to the reducing

substance, 2-ketogluconate, was used originally by Haynes (1951) to distinguish between various pseudomonads. Cowan (1955) showed it was a useful test for *Aerobacter* (*Klebsiella*), since almost all strains of *A. aerogenes* and *A. cloaceae* gave a positive reaction. We have confirmed this, and found it to be a simple, quick, practical method of differentiating between soft-rot coliforms and the very similar common saprophyte *A. cloaceae*, since the soft-rot coliforms invariably give a negative reaction.

TABLE 1. *Biochemical reactions of organisms used in pathogenicity tests*

	Strain reference number												
Biochemical test	SR 2/1	HT/1	QB 4	123	14 B	340	312	377	H 2	EP 3	370	A 81 V	398
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Pectate gel	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	−	−	−	−	−	−	−	+	+	+	−	−	+
Hydrogen sulphide	−	−	−	+	+	−	+	−	+	+	+	−	+
Gluconate test	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+
M.R.	+	+	+	−	−	−	+	−	−	−	−	−	−
V.–P.	−	−	−	+	+	+	−	+	+	+	+	+	+
Gas from glucose	+	+	+	−	+	+	+	+	−	+	−	−	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from lactose	+	+	+	+	+	+	+	+	+	(+)	+	+	(+)
Acid from maltose	+	+	+	+	−	−	+	+	−	−	−	−	−
Acid from salicin	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid in ethyl alcohol agar	−	−	−	+	+	−	+	+	−	+	+	−	−

	399	402	39	H 25	H 35	H 904	C 1	C 5	119 V	B 1	74 V	1172
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Pectate gel	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	−	−	−	−	−	−	−	−	−	+
Hydrogen sulphide	+	+	−	−	+	+	−	−	−	−	−	+
Gluconate test	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+
M.R.	−	−	+	−	−	+	+	+	+	+	+	−
V.–P.	+	+	−	+	+	−	−	−	−	−	−	+
Gas from glucose	+	+	+	+	+	+	+	+	−	+	−	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	+	+
Acid from sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Acid from lactose	(+)	(+)	+	+	+	+	+	+	+	+	+	+
Acid from maltose	−	−	+	+	+	−	+	+	−	+	−	+
Acid from salicin	+	+	+	+	+	+	+	+	+	+	+	+
Acid in ethyl alcohol agar	−	−	−	−	+	−	−	−	−	−	−	+

In gelatin row and pectate gel row; + = liquefaction.
In nitrate row; + = nitrites present.
In lactose row; (+) = acid production delayed at least 4 days.

Failure to produce small amounts of gas from sugars was confined to those cultures considered to be *P. carotovorum* var. *aroideae*. Although not a consistent biochemical reaction, since many gas-forming strains are well known to lose this ability after about a year in culture, we still consider that it is of use as a practical diagnostic characteristic when identifying newly isolated cultures.

Variable results have been reported for the methyl red and Voges–Proskauer tests, no doubt due to the use of different media and times of incubation before testing. For example, Burkholder & Smith (1949) reported the reactions of *P. carotovorum* and *P. carotovorum* var. *atrosepticum* as M.R. positive, V.–P. negative, when grown in ordinary glucose–phosphate–peptone for an unspecified number of days at 27° C.

On the other hand, Jones (1950) reported that all strains of soft-rot coliform bacteria were M.R. negative, V.–P. positive, but he preferred to use O'Meara's (1931) fumarate medium, incubating at 25° C. for 5 days. Taylor (1951) studied the M.R. and V.–P. reactions of eighteen soft-rot isolates, comparing ordinary glucose–phosphate–peptone with a medium claimed to be superior by Smith, Gordon & Clark (1946), and also O'Meara's fumarate medium. He concluded that the Smith, Gordon & Clark medium was useless for tests with soft-rot organisms, while with regard to the fumarate medium he considered that 'reliance should not be placed on its exclusive use, as was done by Jones (1950) or a valuable differential criterion may be lost', because the presence of fumarate favoured the production of acetylmethylcarbinol too strongly. Our results in ordinary glucose–phosphate–peptone incubated for 5 days at 26° C. showed that while all strains of *P. carotovorum* var. *atrosepticum* were M.R. positive, V.–P. negative, the great majority of the other isolates were M.R. negative, V.–P. positive, although strains such as 312 and 119V behaved like var. *atrosepticum*.

It has long been known that the maltose fermentation reaction is a variable character among the soft-rot coliforms, and various workers have attempted to use this feature for separation of species. For example, Dowson (1941) stated that *P. carotovorum* did not ferment maltose, while *P. carotovorum* var. *atrosepticum* did so with the formation of acid and gas. It is now generally recognized that this is incorrect, and that maltose fermentation is of little value in bacterial characterization. This is confirmed by our results, but it is noteworthy that all the *P. carotovorum* var. *atrosepticum* isolates did produce acid from maltose, while of the other organisms only strains 123, 312, 377, H25, H35 and 1172 fermented maltose. A similar situation is found with regard to acid formation from ethyl alcohol. For instance, Dowson (1957) stated that both *P. carotovorum* and *P. carotovorum* var. *aroideae* formed acid in ethyl alcohol agar, while *P. carotovorum* var. *atrosepticum* did not. This is generally true, but there are certain inconsistencies, for strains H2, 340, A81V, 398, 399 and 402 which do not form acid in ethyl alcohol agar cannot be considered as forms of the variety *atrosepticum*. In further experiments we found that cultures which produced acid in ethyl alcohol agar also formed acid in 5% ethyl alcohol-peptone (Bacto) broth (Noble & Marshall, 1952), but the ethyl alcohol beef-extract peptone broth recommended as a differential medium by Burkholder & Smith (1949) gave variable results. Microtests (Clarke & Cowan, 1952; Cowan, 1953) using washed suspensions of organisms grown on meat infusion agar, were carried out in buffered 5% ethyl alcohol with bromothymol blue indicator. These showed that acid production in ethyl alcohol alone was perfectly correlated with acid production in ethyl alcohol agar and ethyl alcohol peptone broth which indicated that the acid was not formed from other substances present in these media.

Most strains produce acid from lactose rapidly, but there are a few notable exceptions which are slower in this respect; these are EP3, 398, 399 and 402. Late lactose fermenters such as *E. carotovora* f.sp. *parthenii* (Starr, 1947), (398), *E. chrysanthemi* (Burkholder, McFadden & Dimock, 1953), (399), and the organisms

causing slow wilt of carnation described by Lelliott (1956), (402), are now well known, but they are much less common than the rapid lactose fermenters. A similar organism isolated from *Philodendron* (EP3) was named *E. chrysanthemi* var. *philodendri* by Miller (1956).

CONCLUSION

Because of the variability of the biochemical reactions, isolates capable of producing potato black-leg at both low and high temperatures, or at higher temperatures only, cannot be distinguished from one another with absolute certainty by biochemical tests alone. However, the tests give an indication of potential pathogenic reactions. The distinguishing tests we tentatively propose are given in Table 2, together with a suggested division into biochemical varieties.

TABLE 2. *Biochemical classification of* Pectobacterium carotovorum *and varieties*

Organism	Gas from glucose	Acid from glucose	Acid from lactose	Acid from maltose	Acid from ethyl alcohol	M.R.	V.–P.	Indole
1. Anaerogenic group								
High-temperature organisms. *P. carotovorum* var. *aroideae*	−	+	+	d*	d	d*	d*	d*
2. Aerogenic group								
(i) Give delayed fermentation of lactose. Indole test invariably +ve (high-temperature organisms). *P. carotovorum* var. *chrysanthemi*	+	+	×	−	d	−	+	+
(ii) Give rapid fermentation of lactose.								
(*a*) Organisms usually M.R. −ve, V.–P. +ve; often produce acid from ethyl alcohol (high-temperature organisms). *P. carotovorum*	+	+	+	d	d	d*	d*	d*
(*b*) Organisms usually M.R. +ve, V.–P. −ve; never produce acid from ethyl alcohol (low-temperature organisms). *P. carotovorum* var. *atrosepticum*	+	+	+	+	−	+	−	−

Notation used is that of Kauffmann, Edwards & Ewing (1956), viz. + = positive 1–2 days; × = late and irregularly positive (i.e. acid production delayed at least 4 days); − = negative; d = different strains give consistently different reactions. Asterisk: in maltose column = most isolates are negative; in M.R. and V.–P. columns = most isolates are M.R. negative, V.–P. positive; in indole column = most isolates are indole negative.

Few of these suggestions are new, since we consider our findings generally support and reinforce the proposals already made by Dowson (1957). For example, var. *aroideae* is still separated on the basis of failure to produce gas from sugars when first isolated, irrespective of any of its other reactions, but we are well aware that this point of view is open to criticism. Such organisms can produce black-leg only at comparatively high temperatures. The low-temperature isolates correspond with var. *atrosepticum*, and are distinguished by their gas production, rapid fer-

mentation of lactose and maltose, M.R. and V.–P. reactions, and failure to give a positive indole test.

Our only new proposal is to alter the name of the chrysanthemum bacterial blight organism from *P. carotovorum* f.sp. *chrysanthemi* (Dowson, 1957), to *P. carotovorum* var. *chrysanthemi*, and include in this variety the carnation slow wilt organism, the guayule pathogen, and the organism causing bacterial leaf rot of *Philodendron*. This is done on the basis of the delayed lactose fermentation reaction, and it is noteworthy that we have found this delay to occur both in inorganic basal media and lactose-peptone media. Great stress is laid by some bacteriologists on the significance of this reaction, and in fact the genus *Paracolobactrum* Borman *et al.* has been established on this criterion; it still appears in Bergey's *Manual of Determinative Bacteriology* (7th edition, 1957). Although we cannot agree that separate genera can be erected on the basis of a single biochemical reaction, we think we are justified in creating a new variety, especially as this group of organisms also gives a positive test for indole. We feel that the diagnosis of soft-rot coliform bacteria should be based as far as practicable on biochemical reactions, and not on host range characteristics, especially as the host ranges of the numerous soft-rot variants have never been completely determined. This is understandable, since most plant pathologists do not have time or facilities for making large-scale inoculations on a range of host plants. Again, as we have shown in the experiments reported in Graham & Dowson (1960), it is not sufficient to draw conclusions from the results of a single pathogenicity trial carried out at any convenient but arbitrarily selected temperature. A range of temperatures should be employed, otherwise the results can be readily misinterpreted. Furthermore, experience has shown that naming an organism solely on the host specificity is commonly of little practical use, because it fails to take into account those from other host plants or from soil, avirulent strains, and those of unknown origin.

At this stage in the study of soft-rot coliforms it is essential to compare the biochemical reactions of as many organisms as possible, to establish firmly a practical biochemical classification on quantitative lines, and at the same time, to investigate the mechanism of pathogenicity.

REFERENCES

Burkholder, W. H., McFadden, L. A. & Dimock, A. W. (1953). A bacterial blight of chrysanthemums. *Phytopathology*, **43**, 522.

Burkholder, W. H. & Smith, W. L. (1949). *Erwinia atroseptica* (van Hall) Jennison and *Erwinia carotovora* (Jones) Holland. *Phytopathology*, **39**, 887.

Clarke, P. H. & Cowan, S. T. (1952). Biochemical methods for bacteriology. *J. gen. Microbiol.* **6**, 187.

Cowan, S. T. (1953). Fermentations: biochemical micromethods for bacteriology. *J. gen. Microbiol.* **8**, 391.

Cowan, S. T. (1955). Biochemical tests for bacterial characterization. II. Gluconate test. *Inter. Bull. bact. Nom. Tax.* **5**, 141.

Cowan, S. T. (1956). Taxonomic rank of Enterobacteriaceae 'Groups'. *J. gen. Microbiol.* **15**, 345.

Dowson, W. J. (1941). The identification of bacteria commonly causing soft-rot in plants. *Ann. appl. Biol.* **28**, 102.

DOWSON, W. J. (1957). *Plant diseases due to bacteria.* Cambridge University Press.

GRAHAM, D. C. & DOWSON, W. J. (1960). The coliform bacteria associated with potato black-leg and other soft rots. I. Their pathogenicity in relation to temperature. *Ann. appl. Biol.* 48, 51.

HAYNES, W. C. (1951). *Pseudomonas aeruginosa*—its characterization and identification. *J. gen. Microbiol.* 5, 939.

JONES, D. RUDD (1950). On the nomenclature and identity of the coliform soft-rot bacteria. *Trans. Brit. mycol. Soc.* 33, 73.

KAUFFMANN, F., EDWARDS, P. R. & EWING, W. H. (1956). The principles of group differentiation within the Enterobacteriaceae by biochemical methods. *Inter. Bull. bact. Nom. Tax.* 6, 29.

LELLIOTT, R. A. (1956). Slow wilt of carnations caused by a species of *Erwinia*. *Plant Path.* 5, 19.

MASSEY, A. B. (1924). A study of *Bacillus aroideae*, Townsend, the cause of soft rot of tomato, and *B. carotovorus* Jones. *Phytopathology*, 14, 460.

MILLER, H. N. (1956). In *Annual Report of the Agricultural Experiment Stations, Florida, for the year ending 30 June* 1956. (Abstr. in *Rev. appl. Mycol.* 36, 808, 1957.)

NOBLE, M. & MARSHALL, M. (1952). A note on black-leg of potato. *Plant Path.* 1, 134.

O'MEARA, R. A. Q. (1931). A simple, delicate and rapid method of detecting the formation of acetylmethylcarbinol by bacteria fermenting carbohydrates. *J. Path. Bact.* 34, 401.

SMITH, N. R., GORDON, R. E. & CLARK, F. E. (1946). Aerobic mesophilic spore-forming bacteria. *Misc. Publ. U.S. Dep. Agric.* no. 559, p. 18.

STARR, M. P. (1947). The causal agent of bacterial stem and root disease of guayule. *Phytopathology*, 37, 291.

TAYLOR, C. B. (1951). The soft-rot bacteria of the coli-aerogenes group. *Proc. Soc. appl. Bact.* 14, 95.

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Control of *Rhizoctonia solani* on potato by disinfection of seed tubers with organo-mercury compounds

by

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CONTROL OF *RHIZOCTONIA SOLANI* ON POTATO BY DISINFECTION OF SEED TUBERS WITH ORGANO-MERCURY COMPOUNDS

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Summary, Résumé, Zusammenfassung, p. 88

Large scale commercial disinfection of seed potatoes by dipping in fungicidal solutions was carried out in Scotland on an experimental basis prior to 1939. This was done to control black scurf caused by *Rhizoctonia solani (Corticium solani)* and other surface-borne diseases including dry rot *(Fusarium caeruleum)*, skin spot *(Oospora pustulans)* and common scab *(Streptomyces scabies)*. More recently the process has been mechanised, and large quantities of potatoes are washed free from soil and disinfected each year, mostly for export to African countries. Although the fungus *R. solani* can be found in almost all Scottish soils, and sclerotia often occur on potato tubers, the organism rarely causes a disease of the potato plant under Scottish environmental conditions, except occasionally in warm summers when the stem canker phase occurs. However, in the African potato growing areas the organism can be very destructive, causing death of sprouts or a severe wilt disease of older plants. Therefore, the importation authorities have laid down regulations to prevent its continuing introduction. In these circumstances the value of disinfected seed is obvious, and to study the effects of fungicidal dipping solutions, experiments have been carried out at Scientific Services for some years.

A comparison of the effectiveness of a number of disinfectant solutions containing various chemicals, including organo-mercury compounds, has already been published (GRAHAM, SRIVASTAVA and FOISTER, 1957). On the basis of failure of treated sclerotia to germinate on potato dextrose agar, it was suggested that, for immersion times of 15 minutes, solutions containing ethoxyethylmercury chloride (EEMC) gave best control, although phenylmercury acetate (PMA) was also reasonably good.

Since these preliminary experiments showed that mercury compounds gave the greatest likelihood of successful control, a further, more extensive series of tests was made using a greater range of organo-mercury products in a search for better disinfection methods. The effectiveness of these products was compared with that of EEMC, PMA and a mixture containing 0,1 % phenol and 0,1 % mercuric chloride. The substances used are listed in TABLE 1; all are commercially available in Great

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Britain except thienylmercury chloride, which was prepared in the laboratory (for method of preparation from thiophene see WHITMORE, 1921).

In these experiments all solutions were made up to contain an equivalent of 100 ppm. of mercury (except in the case of the phenol-mercuric chloride which contained 740 ppm.) so that results were more strictly comparable than in the earlier tests reported by GRAHAM et al. (1957). Representative samples of solutions were checked for mercury content by colorimetric analysis using the diphenylcarbazone method. A suitable amount of an active wetting agent (EOOP, see below) was incorporated in each of the solutions. Tubers bearing sclerotia were dipped at room temperature for 15 minutes and allowed to dry in air. The efficiency of the treatments was judged in the same way as described by GRAHAM et al. (1957), namely by classifying the sclerotia visually into negligible, thin, medium and thick types, and observing the germination of these on potato dextrose agar plates. Streptomycin sulphate (100 ppm.) was incorporated in the agar to prevent the growth of bacterial contaminants which sometimes seem to suppress germination of sclerotia.

TABLE 1. Effect of various mercurial treatments on germination of sclerotia of *R. solani*.

Treatment with *Traitement à Behandlung mit*	Negligible[3]		Thin[4]		Medium[5]		Thick[6]	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1. Phenylmercury acetate (PMA)	60	100	61	85	57	39	51	16
2. Ethoxyethylmercury chloride (EEMC)	80	100	80	98	80	76	88	44
3. Methoxyethylmercury chloride (MEMC)	54	100	72	99	71	85	68	56
4. Phenol-mercuric chloride	40	100	40	60	40	28	40	10
5. Phenylmercurisalicylanilide (PMSA)	60	100	62	97	62	69	74	41
6. Sodium ethylmercurithiosalicylate (Merthiolate)	20	100	20	100	36	78	28	57
7. Methylmercuridicyandiamide (MMDD)	40	100	41	100	38	97	43	84
8. Phenylmercury dimethyl dithiocarbamate (PMDD)	40	38	40	28	30	3	30	0
9. Thienylmercury chloride (TMC)	27	89	26	73	31	32	41	5
10. Control (no treatment)	40	8	40	0	42	0	41	0

(1) = number of sclerotia tested – *nombre de sclérotes examinés – Anzahl der untersuchten Sklerotien.*

(2) = percentage not germinated (to nearest whole number) – *pourcentages de sclérotes n'ayant pas germé (arrondis vers le plus proche nombre entier) – Prozentsätze nicht gekeimt (abgerundet auf die nächste volle Zahl).*

[3] *négligeable – vernachlässigbar*

[4] *petit – klein*

[5] *moyen – mittler*

[6] *gros – gross*

TABLEAU 1. *L'effet de traitements aux solutions mercuriques sur la germination de sclérotes de R. solani*

TABELLE 1. *Wirkung verschiedener Quecksilberbehandlungen auf die Keimung der Sklerotien von R. solani*

The results given in TABLE 1 confirm that EEMC is superior to PMA and phenol-mercuric chloride. MEMC however, was slightly more active than EEMC while Merthiolate had about the same activity as MEMC, although it is unlikely to be used as an alternative owing to its high cost. On the whole PMSA proved to be somewhat less effective than EEMC, although in some individual tests its activity was similar; the reason for this inconsistency is not understood. TMC and especially PMDD had little antifungal activity; this may be because these compounds are relatively insoluble in water, forming suspensions rather than solutions, and therefore cannot diffuse into even the outer layers of sclerotia. MMDD was by far the most effective substance in preventing germination of the sclerotia, but it should not be recommended for use in commercial dipping plants at the concentration employed in this case, as there was evidence that it depressed sprouting in some varieties especially if the treated tubers were stored in a confined space. None of the other treatments appeared to be phytotoxic to tubers of the varieties *Golden Wonder*, *Up-to-Date*, *Kerr's Pink* and *Majestic;* in fact sprouting was even slightly accelerated. However, more extensive experiments are necessary before final conclusions can be drawn regarding phytotoxicity, particularly as the tubers used in these experiments had been lifted and stored for 2 to 3 months before treatment, thereby allowing the skins to harden and the lenticels to seal off. In the commercial dipping process, tubers are washed and treated within 48 hours of lifting to ensure that dry rot and skin spot are controlled, and from time to time „pitting" of the still active lenticels has been observed. This is caused by diffusion of the mercurial poison through the pore, with consequent necrosis of the tissue beneath. In conclusion it is noteworthy that none of the treatments prevented the germination of all sclerotia. This means that dipping cannot replace dressing out; the tubers with large sclerotia must be removed.

EFFECT OF ADDITION OF WETTING AGENTS TO MERCURIAL FUNGICIDES

In the preliminary tests reported in 1957 it was shown that addition of a wetting agent (surface active agent) to solutions of EEMC or the phenol-mercuric chloride mixture increased their effectiveness as fungicides. The wetting agent probably acts in the following way; firstly by releasing any tiny air bubbles trapped in the sclerotia, secondly by allowing the mercurial salt to penetrate into the inner layers of thicker structures and thirdly by affecting cell wall permeability, allowing the mercurial to pass into the individual cells more easily. The power of wetting agents to increase the bactericidal activity of mercurials has been known for some years. For instance LILLEY and BREWER (1949) investigated the effect of certain compounds including mercurochrome, phenylmercury nitrate, Merthiolate and mercuric chloride, each with the addition of the wetting agent sodium lauryl sulphate, on three species of bacteria. Whereas the two components alone were not bactericidal, when added together the solutions were markedly so. A large number of wetting agents are available from various manufacturers, the chemical nature and purity of many of these being known. It was decided to investigate five to see which had the most powerful wetting action, and also to discover if there was any relationship between wetting power and capacity

to increase the activity of fungicides. The products were selected for their differing chemical composition. TABLE 2 gives the chemical nature of the ingredients.

TABLE 2. Chemical composition of wetting agents used

1. EOOP	An octylphenolpolyethylene glycol of 92% purity.
2. SDP	Sodium dinonyl phosphate, 35% pure, containing disodium nonyl phosphate and isopropyl alcohol as impurities.
3. SDSS	Sodium dioctyl sulphosuccinate, 20% solution in water.
4. SDBS	Sodium dodecyl benzene sulphonate, 80% pure with sodium sulphate as impurity.
5. STS	Sodium tetradecyl sulphate, 27% solution in water.

TABLEAU 2. *Composition chimique des agents mouillants utilisés*
TABELLE 2. *Chemische Zusammensetzung der gebrauchten Netzmittel*

EVALUATION OF POTENTIAL WETTING POWER BY A PHYSICAL METHOD

A number of physical measurements may be made on solutions of wetting agents to evaluate their potential wetting and penetrating power. These measurements include capacity to reduce surface tension and interfacial tension, and also the so-called sinking time determinations. It has been shown that surface and interfacial tensions do not give a good measure of wetting potential, whereas sinking time methods are more accurate although the physical phenomena involved are not well understood (THOMPSON, 1958). The sinking time procedure of DRAVES and CLARKSON (1931) as modified by THOMPSON (1958) was employed. This involves measuring the time taken for a skein of cotton yarn attached to a weight to sink in the wetting solution. For each test a 5 gram skein of 3/40S Sudan yarn was placed on a 3 gram copper hook attached to a 50 gram lead weight. The lead weight with skein and hook attached was then dropped into a 500 ml measuring cylinder containing the test solution and the time for the skein and hook to sink was recorded in seconds.

Concentrations of wetting agent varying from 0,2% to 0,01% w/v or v/v were made up in soft tap water at 12°C; the percentage concentration of active ingredient in the original product was taken into account when making the dilutions. The wetting agents were all fairly readily soluble, although SDP was a very viscous fluid and small volumes were difficult to measure accurately. With SDBS it was found best to dissolve the solid in a small quantity of hot water before making up to final volume. Solutions of SDP were cloudy and precipitation was sometimes visible after 3 hours standing, while solutions of EOOP and SDSS were occasionally slightly cloudy but no precipitate was visible after 12 hours. All solutions were used on the day of preparation. Sinking times (in seconds) are given in TABLE 3 and are the average of four determinations.

From the results it may be seen that STS possessed the least wetting power and SDP the greatest, while SDSS, EOOP and SDBS were intermediate in activity. It is clear that the majority of wetting agents should be employed at a concentration of not less than 0,05% in soft tap water, although STS is not very active even at this dilution. SDP would probably be effective at 0,02% under these conditions.

TABLE 3. Sinking times (seconds) in various concentrations of wetting agents

Wetting agent *Agent mouillant* *Netzmittel*	Concentration % v/v or % w/v – *concentration en % de volume ou de poids – Konzentration in Volumen- oder Gewichtsprozenten*				
	0,2	0,1	0,05	0,02	0,01
EOOP	3,0	9	29	150	>900
SDP	0,5	2,2	4,3	21	252
SDSS	2,0	3,0	5,3	53	>900
SDBS	2,0	5,2	22	472	>900
STS	2,0	12	176	>900	–

TABLEAU 3. *Durées d'enfoncement (secondes) dans des concentrations diverses d'agents mouillants*
TABELLE 3. *Absinkzeit in Sekunden bei verschiedenen Konzentrationen von Netzmitteln*

EFFECT OF ADDING STANDARD HARD WATER AND FUNGICIDES TO WETTING AGENTS

The presence of salts affects the activity of wetting agents, sometimes greatly reducing their wetting potential. Instead of using soft tap water, various concentrations of the wetting agents were made up in standard hard water (ANON., 1949), and sinking times determined within 5 minutes of mixing and after standing overnight. The results showed that the activity of SDP was greatly reduced by the presence of the calcium and magnesium salts in the standard solution with an associated heavy precipitation. Solutions of the other ionic wetting agents (SDSS, SDBS, STS) were also affected adversely by the hard water with subsequent precipitation and reduction in activity but none to the same extent as SDP. With the non-ionic wetting agent EOOP the solution remained clear, and there was no significant loss of activity.

To test the effect of mercurial fungicides, dilutions of wetting agents were added to EEMC solution containing 200 ppm. mercury, to PMA solution containing 200 ppm. mercury and to the phenol-mercuric chloride mixture all made up in soft tap water. Sinking time determinations were made within 5 minutes of mixing, when no significant reduction in activity was noticed with any of the combinations. On standing overnight, precipitation took place in some of the mixtures, notably in the solutions containing SDP. However, on the whole there was little significant change in activity, except at the 0,02 % and 0,01 % concentrations where some slight reduction was noted. When standard hard water was used to prepare the EEMC solution, there was no decrease in the efficiency of any of the wetting agents above that caused by the hard water alone.

EFFECT OF ADDITION OF WETTING AGENTS TO MERCURIAL DISINFECTANT SOLUTIONS ON GERMINATION OF SCLEROTIA

On the basis of the physical tests it was decided to add each of the five wetting agents, in an amount to give a final dilution of 0,05 % v/v or w/v, to EEMC solutions containing 100 ppm. and 200 ppm. mercury, to PMA solution containing 100 ppm.

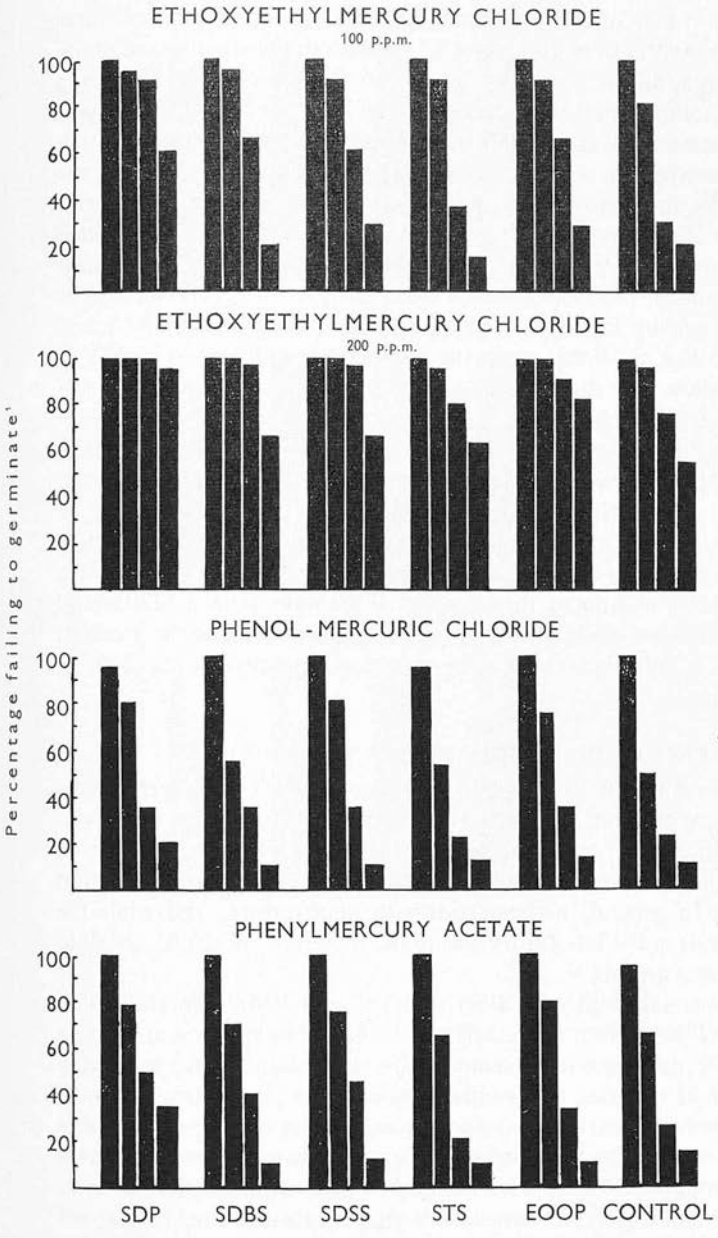

FIG. 1

Histograms illustrating effect of adding wetting agents to mercurial solutions on germination of treated sclerotia of *R. solani*. Each column for each treatment, left to right represents the percentage of negligible, thin, medium and thick sclerotia failing to germinate.

FIG. 1

Histogrammes montrant l'effet de l'addition d'agents mouillants aux solutions mercurielles sur la germination des sclérotes traités de R. solani. Pour chaque traitement, les colonnes de gauche à droite représentent la proportion de sclérotes négligeables, petits, moyens et gros n'ayant pas germé.

ABB. 1

Histogramme zur Darstellung der Wirkung des Zusatzes von Netzmitteln zu Quecksilberlösungen auf die Keimung von behandelten Sklerotien von R. solani. Jede Spalte stellt für jede Behandlung von links nach rechts den Prozentsatz nicht zum Keimen kommender vernachlässigbarer, bzw. kleiner, mittlerer und grosser Sklerotien dar.

[1] *Pourcentage de sclérotes n'ayant pas germé – Prozentsatz nicht zum Keimen kommender Sklerotien.*

mercury, and to the phenol-mercuric chloride mixture, all made up in soft tap water at 16 °C. Tubers bearing sclerotia were dipped for 15 minutes in these solutions, in the mercury solutions without addition of wetting agents and also in solutions of wetting agents alone, to provide suitable controls. Germination of between 20 and 30 sclerotia of each type from each treatment was determined as before. It was found that wetting agents themselves had no effect on sclerotial germination; the rest of the results are summarised graphically by the histograms in FIG. 1. In general, each wetting agent, except STS improved the effectiveness of all dips. For example, when SDP was added to EEMC (at 100 ppm. mercury) the number of thick sclerotia failing to germinate rose from 17% to 60% and of medium sclerotia from 35% to 85%. The next most effective was SDSS followed by EOOP and SDBS. In combination with PMA and phenol-mercuric chloride dips, SDP was again the most effective, followed by SDSS.

The experiments also show that the Draves and Clarkson physical method gives a fairly close correlation between sinking times and capacity to increase fungicidal activity in any particular solution; just as SDP was most effective in the physical determinations in soft tap water, so it was the most effective in the biological tests, while STS was the least effective in both physical and biological experiments. It may be possible, therefore, by measuring sinking times to determine quickly the most active wetting agent, taking into account the dilution at which it whould be used, and the hardness of the water used for making up the solutions. If the water is hard, SDP would be likely to be the least effective, while in soft water its activity would be the greatest, although in most cases it is unlikely to be used in commercial practice owing to precipitation and loss of activity.

EFFECT OF THE TEMPERATURE OF THE DISINFECTANT SOLUTION

The control of *R. solani* was usually found to be poorer under commercial conditions than in laboratory experiments. It seemed likely that the reason for the inconsistency might lie in differences of temperature of the dipping solutions as it is well known that in any disinfection process the temperature of the disinfectant solution greatly affects its action. In general, activity rises with temperature, and while the temperature of the solutions in the laboratory was in the region of 16°–20 °C, the fluid in the commercial tanks was around 6°–7 °C.

To investigate this point solutions of MEMC, EEMC and PMA containing 100 ppm. mercury plus a suitable amount of wetting agent (EOOP) were kept at 6,5 °C – 16,5 °C – 27 °C and 39 °C in a constant temperature water-bath. Tubers bearing sclerotia were dipped for 15 minutes, removed and dried in air, and germination of between 30 and 40 sclerotia of each type was assessed in the usual way, suitable controls being maintained. Placing the sclerotia in a solution of the wetting agent alone at 27 °C for 15 minutes had no appreciable effect on germination, but at 39 °C germination of negligible and thin types was slightly delayed. Results are summarised graphically by the histograms in FIG. 2 – the controls have been omitted for brevity. They demonstrate that temperature greatly affects the antifungal properties of the mercurial solutions. For example about 55% of thick sclerotia treated with MEMC

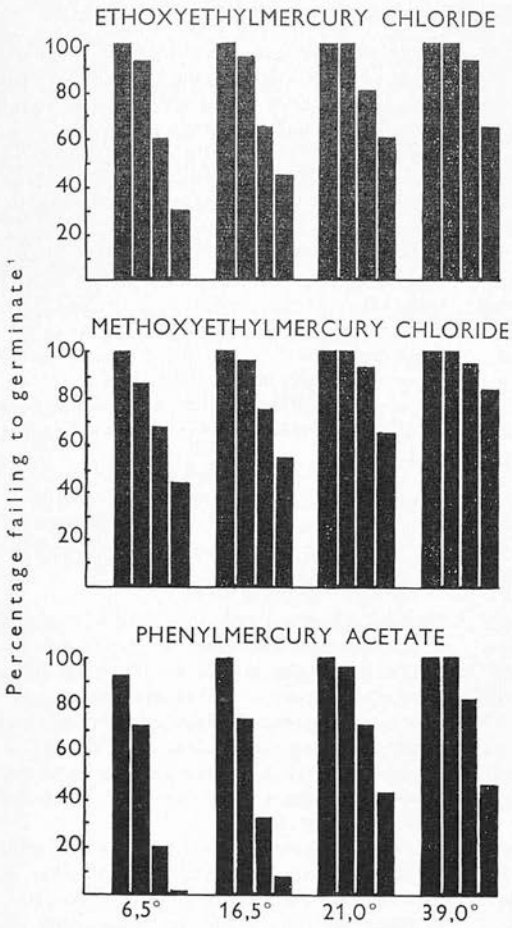

FIG. 2

Histograms illustrating effect of temperature of the disinfectant solution on germination of treated sclerotia of *R. solani*. Each column for each treatment, left to right represents the percentage of negligible, thin, medium and thick sclerotia failing to germinate.

FIG. 2

Histogrammes montrant l'effet de la température de la solution désinfectante sur la germination des sclérotes traités de R. solani. Pour chaque traitement, les colonnes de gauche à droite représentent la proportion ds sclérotes négligeables, petits, moyens et gros n'ayant pas germé.

ABB. 2

Histogramme zur Darstellung der Wirkung der Temperatur der Desinfektionslösung auf die Keimung von R. solani. Jede Spalte stellt für jede Behandlung von links nach rechts den Prozentsatz nicht zum Keimen kommender vernachlässigbarer, bzw. kleiner, mittlerer und grosser Sklerotien dar.

[1] *Pourcentage de sclérotes n'ayant pas germé – Prozentsatz nicht zum Keimen kommender Sklerotien.*

at 6,5 °C germinated but this was reduced to 15 % at 39 °C. A similar trend was found in the experiments with EEMC and PMA. Furthermore, it was noticeable that contamination of the agar plates by saprophytic fungi commonly associated with the sclerotia was greatly reduced by treatment at the higher temperatures. These findings account for the discrepancy in the effectiveness of the commercial process as against the laboratory method and also draw attention to the value of heating the solution to a suitable temperature to obtain the best results. No phytotoxic effect was noticed on the tubers even at the highest temperature, and it may be possible to increase it further with safety. But it must be remembered, as has been explained earlier, that the tubers were lifted two to three months before treatment. More experiments using freshly lifted tubers are necessary before final conclusions can be drawn.

SUMMARY

Although sclerotia of *Rhizoctonia solani* are commonly found on potato tubers in Scotland, the organism rarely causes a disease of any importance under the prevailing environmental conditions. However, it does cause a serious wilt disease in the southern and central areas of Africa which receive imported seed potatoes, and in these circumstances the value of disinfected seed is obvious.

Of a number of possible organo-mercury disinfectants, solutions of soluble substances such as methoxyethylmercury chloride or ethoxyethylmercury chloride containing 100 ppm. mercury have been found to be the most generally suitable, although even these substances do not prevent all sclerotia from germinating. The addition of a suitable wetting agent to the mercurial solution enhances its effectiveness in inhibiting germination or killing of the sclerotia. The potential effectiveness of any wetting agent at any given dilution under a variety of conditions can be predicted by assessing the sinking time by the DRAVES and CLARKSON (1931) method. Increasing the temperature of the dipping solution increases its effectiveness.

None of the treatments described here (except treatment with methylmercuridicyandiamide) appeared significantly phytotoxic to the tubers. However, as the tubers employed in the experiments were lifted two to three months before treatment thereby allowing the skins to harden, and also because the number used was relatively small, definite conclusions on this point cannot yet be reached.

RÉSUMÉ

LA LUTTE CONTRE *Rhizoctonia solani* DANS LA POMME DE TERRE PAR DÉSINFECTION DES PLANTS AVEC DES DÉRIVÉS ORGANO-MERCURIQUES

Bien que la présence de sclérotes de *Rhizoctonia solani* sur les tubercules de pomme de terre soit générale en Ecosse, cet organisme provoque rarement une maladie de quelque importance dans les circonstances existantes. Cependant, il est cause d'un flétrissement grave dans les régions du sud et du centre de l'Afrique qui reçoivent des plants de pomme de terre importés, ce qui rend évidente la valeur de plants désinfectés.

Parmi un certain nombre de désinfectants organo-mercuriques possibles, les solutions de substances solubles telles que le chlorure de méthoxyéthylmercure ou d'éthoxyéthylmercure contenant 0,01 % de mercure se sont trouvées les plus généralement appropriées, bien que même ces produits ne puissent empêcher tous les sclérotes de germer. L'addition d'un mouillant approprié à la solution mercurique augmente son efficacité en ce qui concerne l'inhibition de la germination ou la destruction des sclérotes. L'efficacité possible de tout agent mouillant à n'importe quelle dilution et sous des conditions diverses, peut être déterminée d'avance en observant la durée d'enfoncement selon la méthode de DRAVES et CLARKSON (1931). L'augmentation de la température de la solution où l'on plonge les tubercules renforce son activité.

Aucun des traitements décrits ici (excepté celui au dicyandiamide de mercure) ne présenta de phytotoxicité sensible aux tubercules. Pourtant, les tubercules utilisés dans les essais ayant été récoltés deux ou trois mois avant le traitement, ce qui avait permis à la peau de se durcir, et vu le nombre assez réduit de tubercules observés, on ne peut encore tirer de conclusions définitives à ce sujet.

ZUSAMMENFASSUNG

DIE BEKÄMPFUNG VON *Rhizoctonia solani* IN KARTOFFELN DURCH DESINFEKTION DES PFLANZGUTS MIT ORGANISCHEN QUECKSILBERVERBINDUNGEN

Obwohl Sklerotien von *Rhizoctonia solani* allgemein auf Kartoffelknollen in Schottland vorkommen, verursacht dieser Organismus unter den vorherrschenden Umweltbedingungen hier nur selten schwerere Krankheiten. Dagegen ruft er eine ernstliche Welkekrankheit in den südlichen und Zentralgebieten Afrikas, wo Saatkartoffeln eingeführt werden hervor, und unter diesen Umständen ist der Wert desinfizierten Saatguts offensichtlich.

Von einer Anzahl in Frage kommender organischer Quecksilber-Desinfektionsmittel erwiesen sich Lösungen löslicher Substanzen, wie Methoxyäthylquecksilberchlorid oder Äthoxyäthylquecksilberchlorid, enthaltend 100 Millionstel Teile Quecksilber, im allgemeinen am geeignetsten, obwohl auch diese Stoffe nicht alle Sklerotien am Keimen hindern. Der Zusatz eines geeigneten Netzmittels zu der Quecksilberlösung erhöht die keimverhütende oder tötende Wirkung auf die Sklerotien. Die potentielle Wirksamkeit eines Netzmittels bei einer bestimmten Verdünnung unter verschiedenen Bedingungen lässt sich vorhersagen durch Ermittlung der Absinkzeit nach dem Verfahren von DRAVES und CLARKSON (1931). Die Wirkung der Tauchlösungen wird durch Erhöhung von deren Temperatur gesteigert.

Keine der hier beschriebenen Behandlungen (ausser der Behandlung mit Methylmercuridicyandiamid) erwies sich den Knollen gegenüber als merklich phytotoxisch. Da jedoch die für die Versuche verwendeten Knollen zwei oder drei Monate vor der Behandlung gerodet worden waren, so dass eine Härtung der Schale eintreten konnte, und ferner die Versuche nur mit einer relativ geringen Anzahl durchgeführt wurden, lassen sich diesbezüglich noch keine endgültigen Schlussfolgerungen ziehen.

REFERENCES

ANONYMOUS (1949): Specifications and methods of analysis for certain insecticides and fungicides. H.M.S.O., London. Ministry of Agriculture, Fisheries and Food. *Techn. Bull.* No. 1, pp. 30.

DRAVES, C. Z. & R. G. CLARKSON (1931): A new method for the evaluation of wetting agents. *Amer. Dyst. Rep.* **20**, 109–115.

GRAHAM, D. C., S. N. S. SRIVASTAVA & C. E. FOISTER (1957): The control of *Rhizoctonia solani* on potato. *Plant Path.* **6**, 149–152.

LILLEY, B. D. & J. H. BREWER (1949): Bactericidal effect of mercurials with surface active agents. *Abs. Soc. Amer. Bact., 49th Gen. Meeting*. 27.

THOMPSON, C. C. (1958): The efficiencies of wetting agents used in agricultural sprays. *J. Sci. Food Agric.* **9**, 650–657.

WHITMORE, F. C. (1921): Organic compounds of mercury. Amer. Chem. Soc. Monograph; New York, The Chemical Catalog Co., 333.

EXPERIMENTS ON THE CONTROL OF BLACK-LEG DISEASE OF POTATO BY DISINFECTION OF SEED TUBERS WITH MERCURY COMPOUNDS AND STREPTOMYCIN

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INTRODUCTION

Black-leg disease of potato, caused by the bacterium *Pectobacterium carotovorum* var. *atrosepticum (Erwinia atroseptica)* is very widespread in potato stocks in Scotland, and indeed occurs wherever the potato is grown. However under Scottish conditions the disease rarely reaches epidemic proportions, and in general the rate of tuber transmission is low, so that no special attempts have been made to control the disease other than by roguing crops to the tolerances permitted under the Potato Certification Scheme. In recent years an increasing quantity of Scottish seed potatoes has been exported abroad to warmer countries such as South Africa, Southern Rhodesia and Israel. The variety mostly exported is Up-to-Date, which appears from field observations to be susceptible to black-leg. One of the frequent features of Scottish seed grown in warmer climates has been the high black-leg content of some crops, sometimes as much as 50%, during their first year of cultivation, although crops grown from seed produced in these countries generally have little black-leg; the reason for this phenomenon is not understood at present. Severe outbreaks have seldom occurred in Israel, but black-leg disease has been observed over many years in crops grown from seed newly imported from Scotland (VOLCANI, 1953). With regard to the warmer countries there seems little doubt now that the causal organisms originate from the seed and not from the soil especially since it has been found that the disease is not caused by soft-rot coliform bacteria indigenous there; the strain isolated from infected potatoes can produce black-leg at relatively low temperatures (below 66°F), and is identical with the strain present in Scotland (GRAHAM and DOWSON, 1960a, b).

Much of the seed exported is now washed free from soil and treated with an organomercurial disinfectant solution on a commercial scale in Scotland. This is done to ensure complete freedom from eelworm cysts, especially *Heterodera rostochiensis*, (MABBOTT, 1960) as well as to control surface-borne fungus diseases including dry rot *(Fusarium caeruleum)*, skin spot *(Oospora pustulans)* (BOYD, 1960) and black scurf

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(Rhizoctonia solani) (GRAHAM, 1960). Because of the serious and continuing outbreaks of black-leg, experiments have been made during the last five years on the control of the disease by disinfecting tubers with mercury compounds, streptomycin preparations and later by using a mixture of the two.

Attempts to control black-leg by tuber disinfection have been reported on many occasions, but most of this work has been done in the United States and Canada where cut seed is widely used and where treatment is usually carried out a short time before planting. Antibiotics, particularly streptomycin, have often been employed (for example see BONDE and DE SOUZA, 1954; BONDE, 1955) but formaldehyde (BONDE, 1950) and organo-mercurials including Semesan bel have also been tried (ROBINSON, AYERS and CAMPBELL, 1960).

In general, the results have not been very instructive or promising, partly because the development of black-leg is so sporadic that it has often proved impossible to plan experiments to ensure that a definite result will be obtained even over several years. Blackleg occurred in small amounts in many instances in spite of seed treatment.

In our experiments whole (uncut) seed taken from infected crops was used, and the seed was transported by rail and cargo boat from Edinburgh to Rehovot and Beit Dagan. The tubers were therefore subjected to the changing environmental conditions during transport which may in some way effect the viability of the bacteria or decrease the natural resistance of the tuber to the development of black-leg. When planted in Israel they were of course also exposed to conditions very different from those in Scotland, and it was hoped that outbreaks of black-leg would occur so that the effect of the treatments could be judged more easily.

MATERIALS AND METHODS

In the experiments, three varieties were used, namely *Majestic*, *Epicure*, and *Up-to-Date*, all of which are known to be susceptible to black-leg from field observations. Crops were selected which had a black-leg content of 3% or over at inspection time (mid-July to mid-August). Tubers were drawn at random from the bulked material at normal lifting time in October and placed in bags for storage in a cool airy shed. No special selection of tubers from black-leg affected plants was made, as the process was meant to simulate commercial practice as far as possible. Furthermore, experience has shown that just as many black-leg infected plants are produced from tubers taken from "healthy" plants as those from obviously diseased plants, but why this occurs is not yet fully understood (CONROY, 1952; GRAHAM, unpublished). As soon as possible after bagging, tubers were taken to Edinburgh, washed free from soil and portions treated with the disinfectants. After disinfection they were air dried, then placed in sprouting trays and stacked in an airy frost-proof shed until time for shipping to Israel in early or mid-December. For transportation tubers were packed in 25 lb. lots in stout, ventilated cardboard cartons and the journey by rail and cargo boat usually took 4–5 weeks. On receipt at Rehovot or Beit Dagan, some of the above samples as yet untreated were disinfected and dried, and the tubers planted as soon as

possible in replicated plots on the Research Station. Tubers washed in water only were used as controls.

Three chemical treatments were used, namely a mercurial, streptomycin, and (in two instances) a mixture of the two. The tubers remained in the solutions for 15 minutes at room temperature (16°–20°C). In the case of Experiment 1 (1955–56), streptomycin was used at a concentration of 100 p.p.m. but in all other experiments the concentration was 200 p.p.m. Also in Experiment 1, a solution of mercuric chloride containing 740 p.p.m. mercury was used, and the tubers were rinsed in water before drying to reduce the danger of phytotoxicity. In the other Experiments 3, 4 and 5 the mercurial was ethoxyethylmercury chloride (EEMC) at a concentration of 100 p.p.m. mercury, but in this case the substance was allowed to dry on the tubers. The streptomycin solution and the mixtures of EEMC and streptomycin were also allowed to dry on the tubers. Mercuric chloride, streptomycin and EEMC are known to be actively toxic to the black-leg organism. All the disinfectant solutions contained the non-ionic surface-active agent (wetting agent) octylphenolpolyethylene glycol at 200 p.p.m. concentration to increase their wetting and penetrating action (GRAHAM, 1960).

The tubers were planted about the middle of January in sandy loam during the first four years of the experiments and in a clay loam for the fifth year. The fields were irrigated regularly by overhead sprinklers from the beginning of April until harvest time, but irrigation was also applied during the winter season whenever intervals between successive rains exceeded a period of 12–14 days. Average air temperatures (in the shade) at Rehovot and Beit Dagan ranged between 10–15°C at night to 15–20°C during the day in the winter months but reached 20°C. at night rising to 25°C and occasionally 30°C during the day in spring and at the beginning of summer. These temperatures are very much higher than those reached in Scotland in spring and summer.

As soon as plants emerged, they were examined for the appearance of black-leg, and observations were made periodically throughout the whole of the growing season, since black-leg can develop at any stage of growth. Representative samples of infected plants were checked for the presence of the black-leg organism by the usual isolation and identification methods. Incidental notes were also made on establishment and the presence of diseases other than black-leg.

RESULTS OF DISINFECTION EXPERIMENTS

The results of seed disinfection on black-leg control are summarised in the TABLE. Only in the final experiment (1959–60) did an "epidemic" of black-leg occur in the untreated control, so that the effect of the treatments could be judged accurately. This shows that in general Scottish seed planted in warmer countries does not necessarily give rise to large numbers of black-leg affected plants. The results of the 1959–60 experiments indicate that when an outbreak does occur with untreated seed, treatment with EEMC or streptomycin or both can reduce the amount of black-leg significantly, but that it will certainly not eliminate the disease. When only small amounts

TABLE. Effect of disinfecting whole seed tubers with mercurials and streptomycin on the incidence of potato black-leg disease

Date and number of experiment	*Variety*	*Treatment in*	Treatment – *Behandlung* – *traitement*							
			Streptomycin		Mercurial		Streptomycin plus mercurial		Control (washed only)	
			Nr. of tubers planted	% of plants infected	Nr. of tubers planted	% of plants infected	Nr. of tubers planted	% of plants infected	Nr. of tubers planted	% of plants infected
1. 1955/56	Majestic	Scotland	250	3,6	200	0,0	–	–	250	4,0
2. 1956/57	Majestic	Scotland	184	0,0	–	–	–	–	193	1,0
		Israel	175	1,7	–	–	–	–	–	–
3. 1957/58	Epicure	Scotland	198	2,5	194	12,4	–	–	155	5,8
		Israel	163	11,0	–	–	–	–	–	–
4. 1958/59	Up-to-Date	Scotland	396	0,25	397	0,0	409	0,0	407	0,2
		Israel	499	0,0	473	0,0	356	0,0	–	–
5. 1959/60	Up-to-Date	Scotland	359	12,3	396	7,3	349	6,9	406	21,2
		Israel	385	8,8	400	4,0	388	7,7	–	–
Totals			2.609	4,3	2.060	3,3	1.502	3,6	1.411	7,7

Datum u. Nummer des Versuchs	*Sorte*	*Behandlung in*	*Streptomycin*		*Quecksilberverbindung*		*Streptomycin und Quecksilberverbindung*		*Kontrolle (nur gewaschen)*	
			Anzahl der gepfl. Knollen	*% der infizierten Pflanzen*	*Anzahl der gepfl. Knollen*	*% der infizierten Pflanzen*	*Anzahl der gepfl. Knollen*	*% der infizierten Pflanzen*	*Anzahl der gepfl. Knollen*	*% der infizierten Pflanzen*
Date et No. de l'expériment	*Variété*	*Traitement en*	*Streptomycine*		*Composé de mercure*		*Streptomycine et composé de mercure*		*Témoin (seulement lavé)*	
			Nb. de tubercules plantés	*% des plantes infectées*	*Nb. de tubercules plantés*	*% des plantes infectées*	*Nb. de tubercules plantés*	*% des plantes infectées*	*Nb. de tubercules plantés*	*% des plantes infectées*

TABELLE. *Einfluss der Desinfektion von ganzen Pflanzkartoffeln mit Quecksilberverbindungen und Streptomycin auf Schwarzbeinigkeitbefall*

TABLEAU. *L'influence de la désinfection de plants entiers par des composés de mercure et par streptomycine sur l'incidence de la jambe noire des pommes de terre*

of black-leg occur, chemical treatment appears in general to have little effect, and this agrees with observations in North America (BONDE, 1950). In the case of *Epicure* (1957–58), treatment with EEMC in Scotland and with streptomycin in Israel apparently increased the amount of black-leg. We can offer no explanation for this finding but we have seen several cases in commercial crops where seed treated with an organomercurial or streptomycin has apparently given rise to a greater number of infected plants than untreated seed from the same stock.

No phytotoxic effect of the chemical treatment was noted, and establishment and growth of the plants appeared normal. In the case of Experiment 3 with the variety *Epicure*, wilting of non-bacterial origin was very conspicuous in the untreated controls and to a less extent in the treatments using streptomycin alone, but was much reduced by the EEMC treatment. Fungi including *Rhizoctonia solani*, *Fusarium* spp. and *Colletotrichum atramentarium* were isolated from wilted specimens. Non-bacterial wilting was also observed in Experiment 4, using *Up-to-Date*, but only a few plants were affected and the wilting did not seem to be related to any particular treatment.

DISCUSSION

Successful control of tuber-borne diseases by treatment of tubers with disinfectant solutions must depend on the causal organism being on or near the surface of the tuber, because the penetration of the tuber surface with certain chemical compounds is usually very superficial, even with the addition of a wetting agent. This was demonstrated in the case of mercurials, using the variety *Up-to-Date*, by immersing freshly lifted mature tubers in a 0,4% aqueous solution of mercuric chloride plus 0,02% octylphenolpolyethylene glycol for 30 minutes and drying in air. Transverse sections of the tubers were cut by hand, treated with a 2% aqueous solution of ammonium polysulphide and examined microscopically. The ammonium polysulphide precipitates black mercuric sulphide, thereby indicating the depth reached by the mercuric chloride, and it was found that the mercurial had diffused through only the first four or five layers of the periderm cells. A similar result was obtained using a 0,2% aqueous solution of the organo-mercury dye Mercurochrome (sodium dibromoxymercurifluorescein) plus wetting agent, although in this case the depth of penetration could be observed directly because of the staining action of the compound. The cells on the tuber surface are heavily suberized and therefore water-repellent so that it seems quite probable that other chemical substances such as EEMC or streptomycin would be prevented from reaching any greater depth even though streptomycin is usually regarded as having some systemic activity.

There seems little doubt that contamination of the surface tissues of potato tubers with black-leg bacteria does take place since the black-leg organism can be isolated from the surrounding soil when the plants are growing in the field. Furthermore, it has been possible to isolate the organism by plating washings from skin scrapings on a selective medium (such as salicin bile-salt agar described by NOBLE and GRAHAM, 1956) although this has been successful on a very few occasions only. Because of its small size and its active motility the organism may be able to move into regions in the

skin which the disinfectant does not reach, and this may partly account for the failure of surface disinfection to eliminate the disease. It must be remembered, however, that some of the black-leg infection is systemic particularly in those tubers which were attached to stolons of infected plants. The bacteria migrate into the vascular tissue of the heel ends of the tubers, and in some cases at least, remain latent until the following season, when they begin to multiply, attack the mother tuber and finally pass into the stems of the young plant. The presence of these bacteria in the vascular tissue can be demonstrated by plating on selective media, but again their presence is by no means easy to demonstrate perhaps because of their small numbers and the inadequacy of the selective method. Obviously, bacteria within the vascular tissue are well protected from the action of any surface disinfectant solution. Another point which should not be overlooked is the possibility that the organisms in association with the tubers are in a resting physiological state, so that they are not especially susceptible to the toxic action of disinfectants, for it is well known that actively multiplying cells are the most susceptible. Further experiments are necessary to discover if this is an important factor in the failure of disinfection to control the disease.

It is therefore concluded that surface disinfection of whole seed tubers with mercury compounds or streptomycin cannot be said to control black-leg infection. The failure appears to be caused, at least in part, be inadequate penetration of the tuber tissues, and it seems unlikely that control by chemical methods will be achieved until an active systemic bactericide becomes available. No such compound is known at present.

SUMMARY

Black-leg disease of potato, caused by the bacterium *Pectobacterium carotovorum* var. *atrosepticum (Erwinia atroseptica)* is very widespread in potato stocks in Scotland, but under the prevailing environmental conditions the disease rarely reaches epidemic proportions and is not usually considered to be a serious problem. But when seed tubers are exported from Scotland and grown in warmer countries such as Israel, epidemics of black-leg are liable to develop, and attempts have been made to control these outbreaks by surface disinfection of the whole seed with solutions of several chemical substances.

Disinfectants used included mercuric chloride, ethoxyethylmercury chloride (EEMC), streptomycin and a mixture of streptomycin and EEMC. The solutions also contained a surface active agent to increase their wetting and penetrating action.

Tubers taken at random from black-leg infected crops were disinfected soon after lifting, transported from Scotland to Israel by rail and cargo boat, and then further, as yet untreated samples were disinfected. Tubers washed in water only were used as controls. The seed was planted in replicated plots, given overhead irrigation when necessary, and the black-leg content of the subsequent crop was determined.

The results showed that in the case of one experiment where about one-fifth of the control plants was affected, disinfection reduced the amount of black-leg considerably, but did not eliminate the disease. In another experiment, disinfection appeared to increase the amount of black-leg, while in the remaining three experiments the treatment had no significant effect.

It is concluded that treatment of whole tubers with mercurials, streptomycin preparations or mixtures of the two cannot be said to control potato black-leg disease. The failure of surface disinfection is probably caused partly by some of the bacterial infection being systemic in the tuber and partly by the poor penetration of the disinfectants through the suberized surface layers of the mature tuber.

EXPERIMENTS ON THE CONTROL OF BLACK-LEG DISEASE OF POTATO

ZUSAMMENFASSUNG

VERSUCHE ÜBER DIE BEKÄMPFUNG DER SCHWARZBEINIGKEIT DER KARTOFFEL DURCH DESINFEKTION DER SAATKNOLLEN MIT QUECKSILBERVERBINDUNGEN UND STREPTOMYCIN

Die Schwarzbeinigkeit der Kartoffel, verursacht durch das Bakterium *Pectobacterium carotovorum* var. *atrosepticum (Erwinia atroseptica)* ist in den Kartoffelvorräten Schottlands sehr verbreitet, die Krankheit erreicht jedoch unter den herrschenden Umweltbedingungen kein epidemisches Ausmass, so dass keine speziellen Massnahmen getroffen wurden um die Krankheit zu bekämpfen ausser der Auslese aus den Beständen, um auf Grund des "Potato Certification Scheme" (Kartoffelanerkennungsvorschriften) die Anerkennung zu erhalten.

In den letzten Jahren wurden Pflanzkartoffeln in steigendem Masse nach Ländern mit wärmeren Klima – wie Südafrika, Südrhodesien, Israel – exportiert, wo es sich herausstellte, dass in den von diesem Pflanzgut gezogenen Kartoffelbeständen dort im ersten Anbaujahr ernste Ausbrüche der Schwarzbeinigkeit vorgekommen sind.

Infolge der ernsten und kontinuierlichen Natur dieser Ausbrüche wurden Massnahmen getroffen um diese Krankheit durch eine Oberflächen-Desinfektion der ganzen (nicht geschnittenen) Knollen mit Lösungen von verschiedenen chemischen Präparaten bei drei Sorten zu bekämpfen.

Zur Desinfektion wurde Quecksilberchlorid mit 740 p.p.m. Quecksilber, Äthoxyäthyl-Quecksilberchlorid (EEMC) mit 100 p.p.m. Quecksilber, Streptomycin mit 100 und 200 p.p.m., und eine Mischung von Streptomycin mit 200 p.p.m. + EEMC mit 100 p.p.m. Quecksilber angewandt Die Lösungen enthielten auch den Oberflächen-Wirkstoff Octylphenolpolyäthylen-Glykol in einer Konzentration von 200 p.p.m. um das Befeuchtungs- und Eindringungsvermögen zu erhöhen.

Die willkürlich aus den mit Schwarzbeinigkeit befallenen Beständen genommenen Knollen wurden bald nach dem Roden von der anhaftenden Erde reingewaschen, durch das Eintauchen in die Lösung durch 15 Minuten bei Zimmertemperatur (16–20 °C) desinfiziert und an der Luft getrocknet. Dann wurden sie aus Schotland per Eisenbahn und Schiff nach Israel transportiert, was ung. 4–5 Wochen in Anspruch nahm. Nach ihrer Ankunft in Israel wurden weiterhin die nicht behandelten Muster desinfiziert; für die Kontrolle wurden in reinem Wasser gewaschene Knollen benutzt. Das Pflanzgut wurde in Parzellen mit Wiederholungen ausgepflanzt, nach Bedarf einer Beregnung unterzogen und im Bestand wurde der Schwarzbeinigkeitsgehalt bestimmt.

Die Ergebnisse der Desinfektionsversuche sind in der TABELLE zusammengefasst. Nur im Abschlussversuch (1959–1960) zeigte sich ein "epidemisches" Auftreten der Schwarzbeinigkeit in der unbehandelten Kontrolle, so dass die Wirksamkeit der Bekämpfungsmassnahmen genau beurteilt werden konnte. Dies zeigt, dass das in wärmeren Ländern ausgepflanzte schottische Pflanzgut nicht unbedingt eine grosse Anzahl von mit Schwarzbeinigkeit befallenen Pflanzen zur Folge hat. Die Versuchsergebnisse der Jahre 1959–1960 weisen darauf hin, dass im Falle eines epidemischen Auftretens die Behandlung mit EEMC oder Streptomycin oder mit einer Mischung der beiden das Ausmass der Schwarzbeinigkeit stark herabzusetzen vermag, die Krankheit jedoch nicht eliminieren kann. Wenn nur weinige Fälle von Schwarzbeinigkeit auftreten, scheint die chemische Behandlung einen geringen Nutzeffekt zu haben. Im Falle von *Epikur* (1957–1958) erhöhte die Behandlung mit EEMC in Schotland und mit Streptomycin in Israel merklich das Ausmass der Schwarzbeinigkeit, wir konnten für diese Erscheinung jedoch keine Erklärung finden. Es wurde keine phytotoxische Wirkung der Behandlungen beobachtet und sowohl die Entwicklung wie das Wachstum schienen normal zu sein.

Wir kamen zur Schlussfolgerung, dass die Oberflächen-Desinfektion von ganzen Knollen mit Quecksilberpräparaten und Streptomycin, oder mit einer Mischung von beiden nicht als eine Bekämpfung der Schwarzbeinigkeit zu betrachten ist. Das Versagen der Behandlung ist wahrscheinlich darauf zurückzuführen, dass einerseits die bakterielle Infektion in der Knolle teilweise von systemischer Art ist und dass andererseits die Desinfektionsmittel nur schwach durch die verkorkten Oberflächenschichten der reifen Knolle durchdringen können.

D. C. GRAHAM AND ZAFRIRA VOLCANI

RÉSUMÉ

ESSAIS DE LUTTE CONTRE LA JAMBE NOIRE DE LA POMME DE TERRE PAR DÉSINFECTION DES TUBERCULES DE SEMENCE AVEC DES COMPOSÉS DE MERCURE ET LA STREPTOMYCINE

La jambe noire de la pomme de terre, causée par la bactérie *Pectobacterium carotovorum* var. *atroseptícum (Erwinia atroseptica)* est une maladie très courante dans les cultures de pommes de terre en Ecosse, mais elle prend rarement des proportions épidémiques dans les conditions de milieu existantes, de sorte que l'on ne s'est pas efforcé de combattre cette maladie par des mesures particulières autres qu'un arrachage de plantes atteintes suffisant pour satisfaire à la tolérance admise par le "Potato Certification Scheme" (préceptes de certification des pommes de terre).

Ces dernières années, des quantités croissantes de pommes de terre de semence ont été exportées vers des pays plus chauds, tels que l'Afrique du Sud, la Rhodésie du Sud et Israël, et il a été constaté que de graves épidémies de jambe noire pouvaient se produire dans la première année de culture à partir de ces plants. Vu le caractère grave et ininterrompu de ces épidémies, on s'est efforcé de combattre la maladie par désinfection superficielle des tubercules de semence entiers (non coupés) de trois variétés au moyen de solutions de différents produits chimiques.

On utilisa à cette fin le chlorure de mercure à 740 millionièmes de mercure, le chlorure d'éthoxyéthylmercure (EEMC) à 100 millionièmes de mercure, la streptonycine à 100 et 200 millionièmes et un mélange de streptomycine à 200 millionièmes et d'EEMC à 100 millionièmes de mercure. Les solutions contenaient de plus un produit tensio-actif, l'octylphénolpolyéthylèneglycol à la concentration de 200 millionièmes, destiné à rendre les solutions plus mouillantes pour en faciliter la pénétration.

Des tubercules pris au hasard dans une culture atteinte de jambe noire étaient lavés pour les débarrasser du sol adhérent peu après l'arrachage, puis désinfectés par immersion dans les solutions pendant 15 minutes à la température ambiante (16–20 °C) et séchés à l'air. Ensuite, ils étaient transportés d'Ecosse à Israël par chemin de fer et par cargo, voyage durant de 4 à 5 semaines. Arrivés à destination, les échantillons qui n'avaient pas encore été traités étaient désinfectés et des tubercules uniquement lavés à l'eau étaient pris pour témoins. Les plants étaient plantés dans des parcelles à plusieurs reprises, arrosés quand il le fallait et la proportion de plantes atteintes de jambe noire dans la culture qui en résultait était déterminée.

Les résultats des essais de désinfection sont résumés au TABLEAU. Pendant le dernier essai (1959–'60) seulement, il se produisit une "épidémie" de jambe noire dans la culture témoin non traitée, de sorte que l'on put apprécier exactement l'effet des traitements. Cela permet de constater qu'en général, les plants écossais utilisés dans des pays plus chauds ne présentent pas nécessairement de grands nombres de plantes atteintes de jambe noire. Les résultats de la saison d'essai 1959–1960 montrent que lorsqu'il se produit une épidémie, le traitement par l'EEMC, la streptomycine ou la mixture des deux permet de réduire significativement l'atteinte, mais qu'il est incapable d'éliminer complètement la maladie. Lorsque le nombre de plantes atteintes est peu important, le traitement chimique semble produire peu d'effet. Dans le cas de la variété *Epicure* (1957–'58), le traitement par l'EEMC en Ecosse et la streptomycine en Israël sembla augmenter le nombre de plantes atteintes de jambe noire, mais nous ne savons comment expliquer cette observation. Il ne fut constaté aucun effet phytotoxique des traitements chimiques et le développement et la croissance des plantes semblaient normaux.

La conclusion est que la désinfection superficielle des tubercules entiers par des produits mercuriels, la streptomycine ou une association de ces deux formes de traitement ne peut être jugée suffisante pour éliminer la maladie de la jambe noire. L'échec du traitement est probablement dû en partie au fait que l'infection bactérienne du tubercule est emphytique dans une certaine mesure et d'autre part à la pénétration médiocre des désinfectants dans les couches superficielles suberifiées du tubercule mûr.

REFERENCES

BONDE, R. (1950): Factors affecting potato black-leg and seed-piece decay. *Maine Agr. Exp. Sta.* Bull. No. 482.

——— (1955): Antibiotic treatment of seed potatoes in relation to seed-piece decay, black-leg, plant growth and yield rate. *Plant Dis. Reptr.* **39**, 120–123.

BONDE, R., and P. DE SOUZA (1954): Studies on the control of potato bacterial seed-piece decay and black-leg with antibiotics. *Amer. Potato J.* **31**, 311–316.

BOYD, A. E. W. (1960): Fungicidal dipping and other treatments of seed potatoes in Scotland. *Eur. Potato J.* **3**, 137–154.

CONROY, R. J. (1952): Plant disease—Black-leg of Potatoes in New South Wales. The question of seed transmission. *Agric. Gaz. N.S.W.* **63**, 534–536.

GRAHAM, D. C. (1960): Control of *Rhizoctonia solani* on potato by disinfection of seed tubers with organo-mercury compounds. *Eur. Potato J.* **3**, 80–89.

GRAHAM, D. C., and W. J. DOWSON (1960a); The coliform bacteria associated with potato black-leg and other soft rots. 1. Their pathogenicity in relation to temperature. *Ann. appl. Biol.* **48**, 51–57.

——— (1960b): The coliform bacteria associated with potato black-leg and other soft-rots. 2. Biochemical characteristics of low- and high-temperature strains. *Ann. appl. Biol.* **48**, 58–64.

MABBOTT, T. W. (1960): Observations on the development of the potato root eelworm, *Heterodera rostochiensis* WOLL., on the potato tuber, and the importance of such development in the spread of this nematode on washed tubers. *Eur. Potato J.* **3**, 236–244.

NOBLE, M., and D. C. GRAHAM (1956): A selective medium for the isolation of soft-rot coliform bacteria from plant tissue. *Nature*, London. **178**, 1479–1480.

ROBINSON, D. B., G. W. AYERS and J. E. CAMPBELL (1960): Chemical control of black-leg, dry rot and *Verticillium* wilt of potato. *Amer. Potato J.* **37**, 203–212.

VOLCANI, Z. (1953): Black-leg of potatoes in Israel. *Palest. J. Bot., R. Ser.* **8**, 222–223.

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Black Leg Disease of Potatoes

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MOST diseases of plants are caused by fungi and viruses but potato Black Leg is one of the few economically important diseases in temperate climates caused by bacteria. The organism first isolated in Holland and Germany at the turn of the century and now called *Pectobacterium carotovorum* var. *atrosepticum* is a special strain of a great group of bacteria which cause soft-rot diseases of many kinds of plants, such as tomato, arum lily, celery, maize and pineapple, all over the world.

Black Leg is a very widely distributed disease in Scotland, occurring wherever the potato is grown, but in general, losses in individual crops are not usually high although in some cases damage may be greater than is often realised. It has reached as much as 30 per cent. infection on several occasions, and 50 per cent. has been seen from time to time. It is said to be more common in wet than in dry years but in fact Black Leg was almost as extensive in 1959 as in 1958, dry and wet years respectively, although dry conditions tend to check development of the disease.

Severe outbreaks occur quite often in such warm countries as South Africa, Southern Rhodesia and Israel among crops grown from newly imported Scottish seed. The reason for the severity of the outbreaks in these countries compared with those in Scotland is not yet understood, but it may lie in the warmer climate and the fact that crops are irrigated, which could favour disease development.

Symptoms of the Disease

Outbreaks of Black Leg are almost always attributable to planting infected seed tubers. When the tuber sprouts the bacteria carried with it attack the tuber tissue, causing a soft rot, and the organism spreads into the young stems from the rotting tuber. Indeed, sometimes plants may die before or soon after they emerge above the soil, causing blanking, but usually the first signs of the disease occur in plants fairly early in the season when they are several inches high, before the leaves meet in the drills.

These plants are 'hard' and stunted, with pale green or yellowish foliage. The leaves may be stiff and crisp with their margins rolled inwards, but if the weather is dry they become wilted and soft. Around the base of the stem, usually an inch or two above and below soil level, the tissue is black or dark brown but still feels firm. In dry weather the rot is less conspicuous and sometimes cannot be seen on the stem until it is pulled up, when areas of lighter brown dead dry tissue are exposed. After a time, the leaves of affected plants shrivel and turn brown and the plant dies without having produced any tubers.

Black Leg can, however, develop at any stage of growth in the crop and generally appears continuously throughout the season, often reaching a maximum towards its end. In larger mature plants, when the leaves have met across the drills and especially during spells of wet weather, the basal rot is black, wet and soft, and the tissue can easily be crushed

with the fingers. Blackish streaks may sometimes be seen extending up the stem several inches above the basal rot and the plant has a characteristic 'septic' smell. Very often such stems collapse rapidly even without the foliage becoming yellowed.

In many cases it is found that not all the stems of one plant are affected; sometimes only one or two become diseased. If affected stems are cut across an inch or two above the basal rot, a dark brown stain can be seen in the woody tissue beneath the wings. The browned tissue can also be exposed by sharply stripping away a leaf petiole from the stem. Digging up affected plants shows the mother tubers invariably reduced to a rotten, often evil-smelling mass, and it is here that the infection has originated.

Certain other diseases can produce symptoms not unlike Black Leg, particularly so in infection with the fungus causing Stem Canker (*Rhizoctonia solani*) which is not uncommon in Scotland. Here the basal rot is always hard, black, and somewhat shiny, but there is no discolouration of the woody tissue in the stem above the rot. Sometimes a fine white web of the fungus forms a 'collar' on the stem just at or above soil level. This is never seen in Black Leg infection.

The Disease in Tubers

When older plants are affected the bacteria migrate along the underground stems into the heel ends of the newly forming tubers. Here they can pass into the soft flesh and may set up a rapidly spreading wet rot so that the tubers disintegrate in the soil. Usually, however, the disease does not progress so quickly, and the tubers succeed in preventing spread of the bacteria by laying down barriers of corky cells, often leaving a dry blackened sunken area at the heel end. Sometimes this resistance breaks down in storage, particularly in damp, badly ventilated pits, when a rapid soft rot ensues. Many infected tubers survive the winter, and may give rise to Black Leg-infected plants when planted the following season.

Loss from soft rot of tubers in storage is all too common, and whereas not all storage rots are due to Black Leg bacteria, they are a very important cause of rapid, spreading decay. Odd rotting tubers mixed in amongst the bulk of healthy tubers act as foci of infection because they release enormous numbers of organisms into their surroundings and under conditions of high humidity these bacteria spread to healthy tubers. They enter through lenticels, the heel ends or mechanically damaged areas and, in turn, set up the typical soft wet rot. Black Leg bacteria also often accelerate rotting initially caused by the Dry Rot fungus (*Fusarium caeruleum*), Blight (*Phytophthora infestans*) or Gangrene (*Phoma* spp.).

Spread of the Disease

As was mentioned earlier, Black Leg is spread and perpetuated almost entirely by the planting of infected seed. Most of this infection is latent, and the tubers show no external signs of the presence of the organism. A few tubers which have been attached to infected plants may show internal browning of the vascular ring extending from the heel end, but other conditions can cause similar symptoms. Such tubers are, however, no more likely to produce Black Leg-infected plants than tubers which show no internal symptoms.

In most infected crops there is little evidence of Black Leg spreading from plant to plant in the field, but at times secondarily infected stems

can be found, particularly during prolonged spells of wet weather. The bacteria, probably transmitted by rain-splash, usually enter through parts of the stem exposed when old yellow leaves drop off, although occasionally insect feeding lesions and mechanically damaged areas appear to have acted as entry points. There is considerable evidence however, that tubers taken from apparently healthy plants in a crop which includes diseased plants can quite often give rise to as many infected plants as tubers taken from obviously diseased plants. It is believed that this is caused by movement of bacteria through the soil from the infectors to adjacent tubers on healthy plants. In most cases, however, although many tubers carry latent Black Leg infection, only a few such actually give rise to infected plants. At times this latent infection flares up, but the complex of factors which brings this about is not clearly understood yet.

Contrary to the opinion of many observers, recent work by the Department's Agricultural Scientific Services has so far failed to confirm that the Black Leg organism forms a part of the natural bacterial flora of the soil. Although diseased plants and tubers must release enormous numbers of organisms into the soil, it has not been proved that any of these can survive the winter. This further substantiates the view that the main method of carry-over of the bacteria is in association with potato tubers. There have, however, been cases where first year seedlings have become infected with Black Leg when grown in soil which, although not sterilised, has never grown potatoes before. This seems to show that the organism is present in such soils, for it is not carried through the true seed: but again recent work by the Agricultural Scientific Services indicates that the most likely explanation is that the bacteria can persist for long periods in association with the roots of other crop plants without producing any disease. Indeed, the organism has been isolated from outflow water from water-cress beds, and in Japan a very similar organism has been obtained from the roots of healthy brassicae. At present it is impossible to say how important this carry-over is in commercial practice, and many more experiments are necessary before the epidemiology of Black Leg becomes clearer.

Control Measures

For a number of reasons, none of the usual control measures can be regarded as particularly effective against Black Leg. There are, for example, no known immune varieties, although some (e.g., Arran Pilot, Arran Consul, Eclipse, Epicure, Great Scot, Kerr's Pink, Majestic and Up-to-Date) seem more prone to attack than others. At present there is no attempt to breed for resistance to Black Leg, although this may be introduced into future breeding programmes, provided a suitable source for resistance can be found in breeding material.

Because of the spread of the bacteria in the growing crop through the soil and the possibility of contamination from other hosts, roguing out of affected plants, however thoroughly done, cannot be expected to give a complete guarantee that the seed crop is disease-free. However, regular roguing, beginning as soon as symptoms appear and continuing throughout the season (subject, of course, to the Certification Scheme limitations on pre-inspection roguing) should reduce the amount of infection carried by cutting down the amount of bacterial inoculum in the soil. Needless to say, all tubers of affected plants should be dug up and removed from the crop. This will also cut down the development of soft rot in storage, since it is the tubers from obviously diseased plants which usually become actively rotted and which cause secondary

infections in the pit or bag. Cut seed and seed attacked by fungus diseases such as Dry Rot and Gangrene should never be used for planting. Apart from the danger of blanking, tubers attacked by fungal rots are more likely than sound tubers to produce Black Leg-affected plants. This is because the rotting tends to cause a flare-up of any latent Black Leg infection. For this reason, among others, there is a considerable risk in planting brock.

At one time it was hoped that the washing and disinfection of seed with organo-mercury compounds or streptomycin preparations would help to control the disease. Results of disinfection experiments carried out over the last five years with both these chemicals by the Agricultural Scientific Services in collaboration with the Israeli Ministry of Agriculture have, however, proved inconclusive. The failure of the surface disinfection process appears to be owing to the fact that the bacteria do not remain on the tuber surface, but migrate well into the tuber, possibly through lenticels, and remain latent until the following season. Although it cannot be said that Black Leg is controlled by disinfection, this process has great value in controlling other tuber-borne diseases such as Dry Rot, Skin Spot, Black Scurf, Blight and Common Scab.

Further Outlook

The extensive experiments on Black Leg which have been carried out by the Agricultural Scientific Services have underlined the great lack of knowledge on the epidemiology of the disease. It poses complex questions of fundamental importance in the study of plant bacterial diseases in general. It is the Department's policy to continue to study all aspects of the Black Leg problem, but quick answers cannot be expected. Perhaps the best likelihood of successful control would be through the development of a systemic bactericide, that is a chemical substance which, when sprayed on plants or used as a tuber dip, would penetrate into the internal tissues and kill the bacteria within them. No such compound is known at present, and it is not within the scope of the Department's work to attempt to synthesize and test such substances. Meanwhile, by means of refusing to certify growing crops showing more than a limited amount of infection, the Department's Certification Scheme is making a practical contribution towards restricting the impact of this disease on potato production.

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GROWTH CRACKING AND BACTERIAL SOFT ROT IN POTATO TUBERS

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A HIGH incidence of soft rot in potato tubers was noticed at lifting time in many crops grown in the south-east of Scotland in 1962, particularly in the variety Majestic. The symptoms in many cases resembled those of watery wound rot *(Pythium ultimum)*, but there was no sign of phycomycete mycelium in the tubers. Rotting appeared to progress very rapidly and was usually associated with obvious growth cracks. The internal tissues were soft with a brownish black colour and disintegration of the whole tuber soon followed. Many partially infected tubers found their way into storage where they soon decayed and rotting spread to sound tubers in contact with the disintegrated tissue.

Samples of the soft-rotted tubers were tested for the presence of the potato blackleg organism *(Pectobacterium carotovorum* var. *atrosepticum)* by a serological slide agglutination method (Graham, 1963). In almost every tuber tested obvious agglutination occurred, and the presence of the blackleg organism was confirmed by standard bacteriological isolation and identification methods. Other bacteria such as pectolytic pseudomonads and *Bacillus* spp. can cause soft rot in tubers but they played only a minor part in these cases, although they are commonly associated with rotting of tubers which have been merely water-logged.

FIELD OBSERVATIONS

In an experiment, laid down on loam soil at Boghall Farm, Midlothian, in 1962 to investigate the effect of fertilizer application on the growth rates of foliage and tubers, notes were made on the development of growth cracks and of soft rot on the tubers. The variety used was Majestic and the rates of application of a 12:12:18 concentrated complete fertilizer were 2·5 cwt, 5 cwt, 7·5 cwt, and 10 cwt per acre. The original object of the trial necessitated lifting three plants from each plot at fortnightly intervals between planting on May 7 and lifting on October 24.

During July and early August blackleg-infected plants had become widespread and amounted to six per cent of the crop. From mid-August onwards growth cracks were found on many of the larger tubers on both healthy and diseased plants examined at the lifting periods. Soon after development, the crack looked as if it had been the result of pressing the length of a fine-bladed knife into the tuber and this hair-thin straight line subsequently widened into an irregular V-shaped groove. In such tubers it was frequently found that a soft rot had started at one end of a crack or at the junction of two cracks, and spread radially. The rot progressed quickly, completely disintegrating tubers in ten to fourteen days from the first sign of infection. By the ninth and tenth tuber samplings on September 19 and October 3 respectively, it was clear that the number of tubers showing cracks or soft rot or both was directly related to the amount of fertilizer applied and to be most frequent in the larger tubers. At the time of the bulk lifting on October 24 and at riddling a few days later, a count was made of the number of tubers which had to be

discarded because of soft rot and cracking: the results are summarized in Table 1. Cracking was more frequent in the larger tubers and the frequency increased as the fertilizer rate rose. Similarly the proportion of tubers discarded because of soft rot also increased with rate of fertilizer application, but by this time the differences between treatments were rather less striking, probably because many diseased tubers had disintegrated and therefore could not be included in the counts.

TABLE 1

Proportions of tubers with cracks or discarded for soft rot at lifting (graded over 2¼ in. × 1¼ in. riddles)

Fertilizer applied *cwt/acre*	Numbers of tubers examined	Percentage of ware showing cracking	Percentage of seed showing cracking	Percentage of all tubers showing soft rot
2·5	2660	2·05	0·05	3·27
5	2819	2·55	0·44	3·39
7·5	2874	6·08	0·75	3·70
10	2787	7·28	0·42	4·73
Sig. Diff. (P = 0·5)		1·53	0·41	0·92

TABLE 2

Percentage of soft rot or blackleg in tubers of different sizes examined at lifting in two crops of Majestic (graded over 2¼ in. × 1¼ in. riddles)

Tuber size	Field 1		Field 2	
	Number of tubers examined	Percentage soft rot	Number of tubers examined	Percentage blackleg
Ware	946	6·2	1075	8·8
Seed	2007	1·3	3652	3·8
Chats	611	0·3	961	3·5

Further evidence of a relationship between tuber size and the incidence of infection in the variety Majestic, was obtained from two field trials on blight control. In each, the number of infected tubers was counted; the results are given in Table 2. Field 1 was on level heavy land and here most of the rotted tubers bore growth cracks. On the other hand Field 2 was on a steep freely drained slope. This crop contained many blackleg-infected plants and tubers at harvest had the more usual heel end infection. In both cases the larger tubers were the most frequently infected. A relationship between tuber size and disease incidence has been noted with dry rot, pink rot and skin spot where the larger tubers are also more susceptible, whereas with blight the smaller tubers are more commonly attacked (Boyd, 1960).

DISCUSSION

Growth cracking is a feature normally associated with above average rainfall in August and September, when tubers are bulking quickly. Increasing the amount of fertilizer causes an increase in cracking and also in the number of ware-sized tubers at the expense of seed-sized tubers (Simpson and Crooks,

1961), thereby augmenting the incidence of cracking because cracks are more likely to develop in ware than seed. A high incidence in soft rot has also been observed in years such as 1945 and 1948, when rainfall in Scotland was much above average in August and September.

In reports of both these outbreaks (where some association with cracking was also noted) it is stated that soft-rot coliforms were found among the bacteria in samples of rotted tubers, but the organisms were not specifically identified (for details of 1945 outbreak see Bennett, 1946). Suspicion was not directed towards the blackleg organism in particular, partly because of the difficulty of differentiating between soft-rot coliform strains and partly because of the generally accepted belief that soft-rot coliforms were widespread in soils, so that finding the organisms was not unexpected. In the United States, Rosenbaum and Ramsey (1918) and Ramsey (1919) found no evidence that the blackleg organism could survive the winter months in soil, but several workers, notably Leach (1930) and Bonde (1950) have disputed this and the question is not yet resolved. Under Scottish conditions it seems that the blackleg organism does not, in general, form part of the natural bacterial flora of the soil (Graham 1958, 1962), but during the wet weather of August and September 1962 the organism was isolated from soil on a number of occasions in two blackleg-infected crops, both close to and some distance away from diseased plants, by direct plating of soil suspensions on selective media. It seems possible (although it has not been proved) that the blackleg bacterium is a soil invader rather than a soil inhabitant and it is perhaps not surprising that continuing wet conditions greatly assist invasion. In the crop at Boghall there was a high incidence of blackleg particularly early in the season and the diseased plants might have been sources of bacterial inoculum capable of spreading through the soil when it was wet. Large numbers of organisms moving through the soil water could have reached the susceptible tissues exposed in the cracks setting up the typical soft wet rot, which would account for the relationship between cracking and the disease.

The highest fertilizer rate used in the Boghall experiment, namely 10 cwt per acre is about that most commonly applied to commercial crops. The evidence suggests that the not unusual rate of 12 to 15 cwt per acre would have produced an even higher incidence of cracking and soft rot. It has been shown in 33 potato experiments covering 10 growing seasons that the optimum application rate (i.e., the rate which will give the maximum yield) lies around 7 to 8 cwt per acre in south-east Scotland, irrespective of growing conditions (Simpson, 1957; Simpson and Crooks, 1961). In years with a high rainfall towards the end of the growing season, the loss in yield caused by the use of excessive amounts of fertilizer might be partly explained by the increased incidence of cracking and the spread of soft-rot bacteria from infected plants. As the rot starts up to 8 weeks or more before lifting and can destroy tubers very quickly, part of this loss may go unnoticed.

SUMMARY

During 1962 potato crops throughout the east of Scotland suffered heavily from tuber soft rot caused by the potato blackleg organism, *Pectobacterium carotovorum* var. *atrosepticum*. In examining the results of some field experiments where blackleg occurred, it became clear that there was a direct relationship between tuber size and both the appearance of growth cracks and the amount of soft rot. The incidence of soft rot and growth cracks rose as the rate of application of fertilizer increased. It is suggested that blackleg bacteria can spread through soil from infected plants most effectively in a wet season, and the ability of these bacteria to attack tubers on healthy plants directly is greatly

enhanced by the presence of growth cracks. The cracks are caused partly by weather conditions and partly by high rates of fertilizer application.

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REFERENCES

BENNETT, F. T. (1946). Soft rot of potatoes in 1945 crops. *J. Minist. Agric.*, **53**, 56-8.

BONDE, R. (1950). Factors affecting potato blackleg and seed-piece decay. *Bull. Maine agric. Exp. Sta.*, No. 482.

BOYD, A. E. W. (1960). Size of potato tubers and natural infection with blight and pink rot. *Plant Pathology*, **9**, 99-101.

GRAHAM, D. C. (1958). Occurrence of soft rot bacteria in Scottish soils. *Nature, Lond.*, **181**, 61.

GRAHAM, D. C. (1962). Blackleg disease of potatoes. *Scottish Agric.*, **41**, 211-15.

GRAHAM, D. C. (1963). Serological diagnosis of potato blackleg and tuber soft rot. *Plant Pathology*, **12**, 142-4.

LEACH, J. G. (1930). Potato blackleg: the survival of the pathogene in the soil and some factors influencing infection. *Phytopathology*, **20**, 215-28.

RAMSEY, G. B. (1919). Studies on the viability of the potato blackleg organism. *Phytopathology*, **9**, 285-8.

ROSENBAUM, J. and RAMSEY, G. B. (1918). Influence of temperature and precipitation on the blackleg of potato. *J. agric. Res.*, **13**, 507-13.

SIMPSON, K. (1957). The effect of phosphate fertilizers on the potato crop in south-east Scotland. *J. Sci. Food Agric.*, **8**, 297-301.

SIMPSON, K. and CROOKS, P. (1961). Effects of fertilizers on the yield of potatoes in Scotland. *J. Sci. Food Agric.*, **12**, 131-7.

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SEROLOGICAL DIAGNOSIS OF POTATO BLACKLEG AND TUBER SOFT ROT

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SEROLOGICAL methods are widely used for diagnosis of plant virus diseases both in the laboratory and in the field, but the use of antisera in diagnosing plant bacterial diseases has never become popular. Several workers, including Elrod (1942), Stapp (1961) and Okabe and Goto (1956) have studied the serology of the soft-rot coliform bacteria from the point of view of their antigenic structure and relationships between strains, while Novakova (1957) used serology for the rapid differentiation of blackleg bacteria from other organisms growing on isolation plates. In this laboratory, where large numbers of potato stems and tubers are tested annually for the presence of the potato blackleg bacterium, serological methods, particularly the slide agglutination reaction have proved valuable in making quick diagnoses.

PREPARATION OF ANTISERUM

Antisera to typical isolates of the blackleg organism, *Pectobacterium carotovorum* var. *atrosepticum*, can be prepared by injecting heat-killed suspensions of organisms intravenously into rabbits as described in Mackie and McCartney (1962) but sometimes these methods are not particularly successful and the titre is too low. Better results are often obtained by giving 1 ml intramuscular injections of heat-killed bacteria (60°C for one hour) emulsified with Difco Freund Adjuvant (Incomplete) into each hind leg of the rabbit followed 28 days later by a 1 ml " booster " injection of unemulsified preparation given intravenously. Blood is withdrawn 7 days later and if the antibody titre is too low another 1 ml " booster " may be tried. If no further improvement is obtained the rabbit is probably not sensitive enough to the bacterial antigens and another should be used. Satisfactory antisera are diluted 1/10 with 0·85 per cent saline, preserved by adding Merthiolate at 1/10000 and stored in the refrigerator. An advantage of the intramuscular method is that it avoids any toxic action which the antigen preparation might have on rabbits if given intravenously. Although toxicity has not been observed with the blackleg organism, death of rabbits has been reported on several occasions following injections of soft-rot coliforms of the *carotovorum, aroideae* and *chrysanthemi (parthenii)* types.

METHOD OF TESTING

When testing stems for blackleg by slide agglutination, a piece of rotted tissue (about as much as would cover a sixpence) is wrapped in a small square of coarse muslin and two drops of sap are squeezed out with pliers on to each end of a microscope slide. One drop of antiserum is added to the sap at one end and one drop of normal serum to the sap at the other end which then acts as a control. The slide is carefully rocked to and fro to mix the liquids, and if the blackleg organism is present, a flocculent precipitate forms quite quickly in the sap-antiserum mixture. If the stem tissue is rather dry it is helpful to wet

the tissue in the muslin with a few drops of tap water before squeezing – this will usually extract enough bacteria for a satisfactory reaction. Rotten tuber tissue can be tested in the same way, but in this case it is especially important to use material from the advancing edge of the rot, as the tuber tissue behind usually contains large numbers of other bacteria and a satisfactory agglutination may not be obtained. Care should be taken not to confuse suspended particles of plant debris (such as starch grains) with agglutinated bacteria. If there is doubt, microscopical examination under low power should prove conclusive.

SPECIFICITY OF THE AGGLUTINATION REACTION

Antiserum prepared against the blackleg organism will react in slide agglutination tests with certain related bacteria of the *P. carotovorum* and *P. aroideae* types, although some of the reactions are relatively slow and give a precipitate which is more granular in appearance. It does not seem to react with *P. chrysanthemi* types, although definite conclusions cannot be drawn because only five isolates were tested. The results for the number of isolates agglutinated against the number of isolates tested (in pure culture) are as follows: *P. atrosepticum* 56/56; *P. carotovorum* 3/11; *P. aroideae* 7/20; *P. chrysanthemi* 0/5. This means that the serum is not specific for *P. atrosepticum* and since organisms in the other groups are antigenically heterogeneous the antiserum cannot be made significantly more specific by preliminary absorption of the common antibody with cells of these reacting isolates. However, in practice cross-reactions are not very important under Scottish conditions, because the organism which is almost always responsible for blackleg is *P. atrosepticum* (Graham and Dowson, 1960) and the most common coliform responsible for tuber soft rot is also *P. atrosepticum*. This means that a positive reaction with a rapidly forming flocculent precipitate is strongly indicative of the presence of the blackleg organism, and this has been proved repeatedly by subsequent isolation and characterization of the causal organism by standard bacteriological methods. It is noteworthy that on several occasions tuber soft rots have yielded *P. aroideae* types which do not react with *P. atrosepticum* antiserum, but compared with the great number of times *P. atrosepticum* has been found, the former are not very significant and do not greatly detract from the value of the test as a rapid indication of coliform infection. *P. carotovorum* and *P. aroideae types* which do react with *P. atrosepticum* antiserum have not so far been found in Scottish potato material, but no doubt they could be discovered by constant searching and they may be more common in other parts of Britain. Such isolates have been found among organisms received from the U.S.A. and Denmark.

In general, *atrosepticum* antiserum does not react in slide agglutination tests with other members of the Enterobacteriaceae or the Achromobacteriaceae and results for the number of isolates agglutinated against the number of isolates tested were as follows: *Erwinia lathyri* 0/6; *Aerobacter (Klebsiella) aerogenes* 0/4; *Escherichia coli* 0/12; *Serratia marcescens* 0/2; *Achromobacter* spp. 0/3; *Flavobacterium* spp. 0/2. It has given a slight reaction with one strain of *Aerobacter (Klebsiella) cloaceae*, (1/16), but the formation of the precipitate is very slow. In the family Pseudomonadaceae the antiserum does not react with *Aeromonas* spp. (0/3), nor with the great majority of the species of *Psuedomonas*, but two isolates from soil of the *Ps. fluorescens* type were found to give excellent and rapidly forming flocculent precipitates. (Note: pseudomonads often autoagglutinate in saline or normal serum-saline mixtures (" rough " variants), which must be watched for carefully when testing them). This, together with the reaction with *A. cloaceae*, could be a serious drawback,

because these organisms can often be found in large numbers particularly in rotten tuber tissue and are very common in soil. So far, however, members of the *Ps. fluorescens* and *A. cloaceae* groups which have been isolated from tubers in this laboratory have not given reactions, but these findings draw attention to a limitation of the method. It cannot replace standard isolation and identification procedures if the diagnosis is to be made with absolute certainty.

REFERENCES

MACKIE, T. J. and MCCARTNEY, J. E. (1962). *Handbook of Bacteriology,* Edited by Robert Cruickshank. E. and S. Livingstone, Edinburgh, pp. 325-26.

ELROD, R. P. (1942). Serological studies of Erwineae, II. Soft rot group; with some biochemical considerations. *Bot. Gaz.*, **103**, 266-79.

GRAHAM, D. C. and DOWSON, W. J. (1960). The coliform bacteria associated with potato blackleg and other soft rots. I. Their pathogenicity in relation to temperature. *Ann. appl. Biol.*, **48**, 51-7.

NOVAKOVA, JARMILA (1957). A new method of isolation of blackleg pathogens from diseased plants. *Phytopath. Z.* **29**, 72-4.

OKABE, N. and GOTO, M. (1956). Studies on strains of *Erwinia carotovora* (Jones) Holland. I. Antigenic structures of flagella and their relations to pathogenicity and maltose fermentation. *Bull. Fac. Agric. Shizuoka Univ.*, **6**, 16-32.

STAPP, C. (1961). *Bacterial Plant Pathogens.* Oxford Univ. Press, pp. 148-51.

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THE USE OF ORGANO-TIN COMPOUNDS AS POTATO TUBER DISINFECTANTS, PARTICULARLY AGAINST *RHIZOCTONIA SOLANI*

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1. INTRODUCTION

Control of numerous fungus diseases associated with potato tubers including *Rhizoctonia solani* infection has been achieved for many years by disinfection of seed tubers with various chemical compounds, in particular derivatives of mercury. As has been reported previously (BOYD, 1960; GRAHAM, 1960) large-scale treatment of potato tubers within 48 hours of lifting has been carried out commercially in Scotland for several years, mostly using an alkoxyalkylmercury compound as the active ingredient in the disinfectant solution.

Whenever mercury compounds are employed, account has to be taken of their very poisonous nature. In their use for disinfection of seed tubers there are two potential risks for workers at the disinfection plants. On exposure to air, disinfected tubers immediately after treatment give off organo-mercury vapour. This normally dissipates quickly but the storage of disinfected tubers in bulk calls for suitable ventilation arrangements to prevent accumulation of vapour (as well as to facilitate adequate drying of tubers after treatment). The other risk is skin contact with the mercurial solution. This calls for careful design and operation of the processing machinery. There are no mercury-residue hazards to workers planting disinfected tubers or to the consumer of crops grown from such seed. The eating of disinfected tubers themselves at any time would constitute a danger (chemical analyses have shown that even after a period of storage of two months up to 2,0 parts per million (p.p.m.) metallic mercury – mainly in the peel – can be detected in undamaged tubers treated 48 hours after lifting).

There has been no suggestion of mercurial poisoning among workers at the commercial disinfection plants in Great Britain, nor has any case of treated tubers being eaten been reported. But having regard to increasing commercial interest in the use of disinfectant solutions a search for less poisonous chemicals has been continuing and the recently introduced organo-tin compounds seemed promising. The fungicidal properties of these substances were discovered by VAN DER KERK and LUIJTEN (1954) and since then a range of alkyl- and aryltin derivatives has become available for testing. One compound, triphenyltin acetate, has been widely used commercially in agriculture, particularly for controlling potato blight (*Phytophthora infestans*), *Septoria* leaf

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spot of celery and leaf spot of sugar beet caused by *Cercospora beticola* (HARTEL, 1958, 1962; HOLMES and STOREY, 1962). More recently, formulations of triphenyltin hydroxide have come into commercial use (PIETERS, 1961).

Mammalian toxicity studies show that in general organo-tin compounds vary considerably in their properties and some ethyltin derivatives are very toxic (BARNES and STONER, 1958). A great therapeutic disaster following the use of diethyltin diiodide has been reported from France: 102 people died and others were left with permanent neurological damage (ANON, 1958). However as the aliphatic chain length is increased, toxicity falls – thus tributyltin compounds are excellent fungicides and are also much less toxic to mammals. For example, BARNES and STONER (1958) fed rats over a period of three months with tributyltin acetate and produced no symptoms at a level of 50 p.p.m., though at 100 p.p.m. some oedema of the central nervous system was produced. LYLE (1958) made observations on workers at a plant manufacturing organo-tin compounds and found that although tributyltin derivatives could cause skin lesions, these were usually painless and healed without special treatment.

2. SOME PHYSICAL PROPERTIES OF ORGANO-TIN COMPOUNDS USED IN THE EXPERIMENTS

Tributyltin oxide ((TBTO) and tripropyltin oxide (TPTO) are pale-yellow liquids whereas tripropyltin acetate (TPTA) and tributyltin acetate (TBTA) are white, waxy solids. Triphenyltin acetate (TPHTA) is a white to cream powder and diethyloctyltin oxide (DEOTO) and diethyllauryltin oxide (DELTO) are colourless liquids. Dibutyltin dichloride (DBTDC) is a yellow to light-brown solid.

From the point of view of fungicidal activity against sclerotia of *Rhizoctonia solani*, it appears that a very important property of the fungicide is its solubility in water since it is necessary for the compound to penetrate the closely-knit layers of hyphae and then diffuse through the cell membranes to inhibit or kill the organism. This has been shown in the case of organo-mercury compounds where highly insoluble substances such as phenylmercury dimethyldithiocarbamate (PMDD) were found to have no appreciable activity against *R. solani* (GRAHAM, 1960).[1] As no precise information on the water solubility of organo-tin compounds was available this was determined for each of the compounds: excess of the compound was shaken with distilled water for 24 hours at room temperature, the aqueous solution separated by centrifugation and filtration and the concentration of tin determined by colorimetric analysis using the dithiol (dimercaptomethylbenzene) method after destruction of the organic part of the molecule by wet oxidation. Only technical grade substances were available and as these might have contained inorganic tin the results may not be strictly accurate. The results given in TABLE 1 are the average of a number of determinations.

[1] Saturated solutions of PMDD in water at 20 °C contain only about 5 p.p.m. mercury as determined by colorimetric analysis using the diphenylcarbazone method.

TABLE 1. Solubility of organo-tin compounds in water

Concentration of tin in p.p.m. at 20°C (at saturation) – *Konzentration von Zinn in p.p.m. bei 20°C (bei Sättigung) – concentration d'étain à 20°C (à saturation)*							
TPTO	TPTA	TBTO	TBTA	TPHTA	DEOTO	DELTO	DBTDC
473	460	140	120	15	456	143	205

TABELLE 1. *Die Wasserlöslichkeit der organischen Zinn-Verbindungen*
TABLEAU 1. *La solubilité dans l'eau des composés organiques d'étain*

3. TESTS AGAINST RHIZOCTONIA SCLEROTIA ON POTATO TUBERS

In these experiments all solutions or suspensions were made up to contain an equivalent of 100 p.p.m. tin, and representative samples were checked for tin content by colorimetric analysis using the dithiol procedure. To increase wetting and penetrating activity octylphenolpolyoxyethylene glycol wetting agent was incorporated in each of the solutions at 200 p.p.m. concentration (GRAHAM, 1960). As in experiments with organo-mercury compounds, tubers of the variety *Up-to-Date* (from several different areas of Scotland) with sclerotia attached were dipped at room temperature for 15 minutes and allowed to dry in air (the tubers were lifted and placed in boxes 2 days before treatment). The efficiency of the treatments was determined as before (GRA-

TABLE 2. Effect of various organo-tin treatments on germination of sclerotia of *R. solani*

Treatment with *Behandlung mit Traitement avec*	Negligible *Unbedeutend Négligeable*		Thin *Dünn Faible*		Medium *Mittel Moyen*		Thick *Dick Fort*	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Tripropyltin oxide (TPTO)	46	96	62	57	53	40	58	19
Tripropyltin acetate (TPTA)	38	100	31	94	38	54	31	29
Tributyltin oxide (TBTO)	48	94	68	53	46	25	44	18
Tributyltin acetate (TBTA)	64	100	73	93	64	62	79	42
Triphenyltin acetate (TPHTA)	20	100	24	92	23	30	35	16
Diethyloctyltin oxide (DEOTO)	20	100	51	100	39	95	51	89
Diethyllauryltin oxide (DELTO)	37	15	26	8	24	0	20	0
Dibutyltin dichloride (DBTDC)	25	12	22	0	18	0	21	0
Stannous chloride ($SnCl_2$)	29	0	28	0	32	0	29	0
Control (no treatment) – *Kontrolle (unbehandelt) – témoin (aucun traitement)*	30	0	30	0	30	0	30	0

(1) Number of sclerotia tested – *Anzahl der geprüften Sklerotien – nombre de sclérotes testées.*
(2) Percentage not germinated (to nearest whole number) – *Prozent nicht gekeimter Sklerotien (auf die nächste ganze Zahl auf- oder abgerundet) – pourcentage de non germination (exprimé en nombre entier le plus proche).*

TABELLE 2. *Wirkung verschiedener Behandlungen mit organischen Zinn-Verbindungen auf die Keimung der Sklerotien von* R. solani
TABLEAU 2. *Effets de divers traitements aux composés organiques d'étain sur la germination des sclérotes de* R. solani

HAM, SRIVASTAVA and FOISTER, 1957; GRAHAM, 1960) by classifying sclerotia visually into negligible, thin, medium and thick types and observing the germination of these on streptomycin sulphate potato dextrose agar plates.

The results given in TABLE 2 show that TBTA has the same order of activity as ethoxyethylmercury chloride (EEMC) while TPTA is somewhat less active. The corresponding oxides are less effective and are about as active as phenylmercury acetate (PMA) (cf. GRAHAM, 1960). TPHTA, DELTO and DBTDC are of no value as fungicides in this instance, TPHTA probably because of its low water solubility and DBTDC because of its intrinsically low toxicity (VAN DER KERK and LUIJTEN (1954) showed that in general dialkyltin derivatives were poor fungicides). Excellent results were obtained with DEOTO and its activity corresponds with that of methylmercuridicyandiamide (MMDD) which was the most effective organo-mercurial against *R. solani* tested by GRAHAM (1960). Unfortunately MMDD could not be used as a tuber disinfectant because of its phytotoxicity, but DEOTO did not seem to have any deleterious effect on the few tubers used, although more tests would be necessary before final conclusions could be drawn (see below).

4. PHYTOTOXICITY

During the dipping tests, phytotoxic action of the organo-tin compounds was noted, particularly on freshly lifted tubers. An experiment designed to study phytotoxicity was carried out in late October 1960, using hand-washed, freshly lifted tubers of the varieties *Up-to-Date* and *Dunbar Standard*. The haulms of both varieties were burned down with sulphuric acid 28 days before lifting to accelerate skin maturation, which reduces chemical damage as much as possible. However, treatment with TPTO and TPTA caused very severe damage with extensive lenticel pitting and skin necrosis; most of these tubers would have been quite unsuitable for sale. TBTA and TBTO also caused considerable lenticel pitting, but the appearance of the tubers was much better than with TPTO or TPTA and most would have been saleable. TPHTA produced no obvious damage to the tubers, and similarly treatment with methoxyethylmercury chloride (MEMC) and phenylmercury acetate (PMA) (used for comparison) caused no significant effect. There was insufficient DEOTO or DELTO to treat enough tubers for observations to be made.

Sprouting was greatly delayed by the TBTO and to some extent with TPTA and TPTO treatments but TBTA and TPHTA had no significant sprout-depressing activity. MEMC also showed no effect, but PMA almost completely prevented normal sprouting in *Up-to-Date*, causing a curious form of bud proliferation (this effect is not always observed when PMA is applied immediately after lifting and it cannot yet be explained).

To assess any marked effects of organo-tin treatments on growth and yield a small trial was laid down with the *Up-to-Date* tubers mentioned above using 16 tubers per plot replicated four times, planted in mid-April, 1961. Observations were made on emergence, yield and the numbers of tubers in the three grades, ware, seed and chats.

Controls consisted of untreated tubers stored in boxes at lifting time. Emergence was greatly delayed by the PMA treatment as would be expected from the effect on the buds, and some delay was also caused by treatment with TBTO and TBTA. However by the end of June all the tubers in each treatment except PMA had emerged and by mid-July there were no significant differences in the appearance of top growth from any of the treatments except PMA. The plants were allowed to die down naturally and in mid-October the tubers were lifted and weighed, and then graded over $2\frac{1}{4}$ in $\times$ $1\frac{1}{4}$ in (5,18 cm $\times$ 2,64 cm) riddles. The results are shown in TABLE 3.

TABLE 3. Effects of various chemical treatments on yield and numbers of ware, seed, chats (var. *Up-to-Date*)

Treatment *Behandlung Traitement*	Tubers per 32 plants – *Knollen pro 32 Pflanzen – tubercules par 32 plantes*			
	Wt. (lb) *Gewicht (lb) poids (lb)*	Number – Anzahl – nombre		
		ware *Handelsware commerciaux*	seed *Saatgut plant*	chats *kleine Knollen grenaille*
Control – *Kontrolle – témoin*	72	96	189	99
MEMC	$67\frac{1}{2}$	101	160	106
PMA	29	22	130	122
TBTO	$66\frac{1}{2}$	72	184	123
TPTO	$62\frac{1}{2}$	77	101	167
TPHTA	61	58	195	138
TBTA	$56\frac{1}{2}$	69	198	156
TPTA	48	51	164	154
	L.S.D = $12\frac{1}{4}$			

1 lb = 0,4536 kg.
L.S.D. = *G.D. – l'écart minimum.*

TABELLE 3. *Wirkungen von verschiedenen chemischen Behandlungen auf Ertrag und Anzahl Knollen in Handels- und Saatsortierung sowie Anzahl an kleinen Knollen (Sorte* Up-to-Date*)*

TABLEAU 3. *Effets de différents traitements chimiques sur la production et le nombre de tubercules commerciaux, de plants et de grenaille (var.* Up-to-Date*)*

In every case chemical treatment reduced the yield with respect to the control but only with PMA, TBTA and TPTA was the reduction significant. However, only those tubers which were not badly damaged by the TBTO and TPTO dips were planted; if some of them had been used there is a strong possibility that the yields from these treatments would also have been significantly reduced. Every treatment except MEMC also reduced the number of tubers reaching ware size, but in general, there was no increase in the number of seed tubers to compensate for the loss of ware which sometimes occurs following chemical treatment (DE LINT and VAN EMDEN, 1957), although there was a considerable increase in the number of chats.

5. COMPARISON BETWEEN THE EFFECTS OF AN ORGANO-MERCURY AND AN ORGANO-TIN DISINFECTANT IN A FIELD TRIAL

On the basis of the foregoing experiments it was decided to compare the action of a standard mercurial tuber disinfectant, MEMC, with an organo-tin compound on tuber emergence and yield. The effect of the treatments on tuber diseases was also assessed, but again the main object was to observe phytotoxicity, this time on a larger scale and also where a commercial mechanical washing machine had been used to wash tubers. TBTA was finally chosen even though the small-scale trial had indicated it to be liable to cause phytotoxicity, as it is a good fungicide and is relatively cheap, readily obtained and being a white powder soluble in alcohol, easily brought into solution. The trial was carried out using the common variety *Majestic*, and since the particular stock used had very little *Rhizoctonia* infection, the presence of stem canker and the degree of infection with sclerotia on the subsequent crop of tubers was not determined.

Tubers were lifted by spinner in mid-October, 1961 (28 days after burning down the haulms with sulphuric acid), then brought in bulk from the field in bags. The tubers were distributed at random into five equal portions by weight, graded over $2\frac{1}{4}$ in $\times$ $1\frac{1}{4}$ in riddles and the ware and chats discarded. The seed portions were then given the following five treatments within 48 hours of lifting:

A. Boxed only.
B. Washed in a mechanical potato washing machine at 70 lb/in^2 (4,9 kg/cm^2) water pressure, then boxed.
C. Washed as B, dipped 12 min in MEMC solution containing 100 p.p.m. mercury + wetter, then boxed.
D. Washed as B, dipped 12 min in MEMC solution containing 50 p.p.m. mercury + wetter, then boxed.
E. Washed as B, dipped 12 min in TBTA solution containing 100 p.p.m. tin + wetter, then boxed.

Clamp storage was not included among the treatments as the experiment was not designed to compare chemical treatment with normal storage practice in Scotland and for the present purposes the boxed at lifting treatment provided a suitable control.

After treatment the tubers were dried in a warm shed for two days and then stored in a cool shed (minimum temperature 4°C) until April, 1962. The boxes were distributed at random to avoid positional effects and counts of diseased and damaged tubers were made throughout the storage period. Tubers were examined in late March for sprouting, but as the eyes were just beginning to develop, no numerical assessments were made. As far as could be judged using a hand lens, there was considerable eye damage caused by TBTA, but other treatments were apparently normal. A proportion of the tubers was planted to assess emergence and yield.

5.1. Effect of treatment on tuber rots

All tubers in each treatment were counted and damage and diseases classified according to symptoms as dry rot (*Fusarium caeruleum*), gangrene (*Phoma foveata*), soft rot

(various bacteria), blight (*Phytophthora infestans*) and chemical damage. The results are summarised in TABLE 4; mechanically-damaged tubers and the few miscellaneous rots (such as blackleg) have been omitted from the table.

TABLE 4. Effect of various treatments on incidence of tuber rots

Treatment *Behandlung* *Traitement*	Total tubers *Total Knollen* *Totaux des tuberc.*	Percentage – *Prozent* – *pourcentage*				
		Dry Rot[1]	Gangrene[2]	Blight[3]	Soft Rot[4]	Chemical damage[5]
A	385	6,8	20,0	5,6	0,0	–
B	407	4,2	16,5	4,0	0,25	–
C	336	0,3	2,0	5,4	2,0	5,0
D	417	0,0	2,4	4,3	2,0	4,8
E	418	0,25	1,0	4,2	1,0	22,5

[1] *Trockenfäule – pourriture sèche.*
[2] *Phomafäule – gangrène.*
[3] *Phytophthora-Knollenfäule – Phytophthora.*
[4] *Nassfäule – pourriture molle.*
[5] *Schäden durch chemische Verbindungen – dégâts chimiques.*

TABELLE 4. *Wirkung verschiedener Behandlungen auf den Befall mit Knollenfäulen*
TABLEAU 4. *Effet de différents traitements sur l'incidence des pourritures de tubercules*

Gangrene developed to a considerable extent in both the riddled and boxed and also in washed and boxed, although it was somewhat reduced by washing alone. Chemical treatment with both MEMC at the two concentrations and TBTA greatly reduced the disease, TBTA most of all. Likewise, both MEMC and TBTA controlled dry rot, but had no effect on blight, probably because tuber infection was already established before treatment. Soft rot was only slightly increased but the amount of chemical damage resulting from treatment was serious, particularly where TBTA had been used. *Majestic* appears to be a variety rather susceptible to chemical damage and this tendency was exaggerated with the particular stock used in the experiment as the tubers were badly skinned during riddling and mechanical washing, which exposed large areas of tissue to the chemical. The phytotoxic action of TBTA was therefore fully manifested and indeed many tubers were reduced to a brown rotten mass in a few days. It is noteworthy that the rotting was greatly accelerated by pectolytic bacteria of the genus *Pseudomonas* which survived the disinfection, even although *in vitro* tests show organo-tin compounds to be good bactericides. Compared with TBTA, MEMC caused much less damage – a further example of the generally slight to moderate phytotoxic action of this substance.

5.2. Effect of treatments on emergence and yield

Apparently sound tubers were planted in mid-April, 1962 in a 5 × 5 latin square layout, each plot containing 42 tubers, and final emergence counts were made during the first week of July; results are given in TABLE 5.

Greatest emergence was obtained with the boxed only treatment whereas all other

TABLE 5. Effect of treatments on emergence, total yield and yield of ware, seed and chats (yields in lb per treatment)

Treatment *Behandlung Traitement*	Emergence *Auflauf Levée* (%)	Total yield *Gesamtertrag Rendement total*	Ware *Handelsware Commerciaux*	Seed *Saatgut Plant*	Chats *Kleine Knollen Grenaille*
A	94,8	457	287	159	11
B	78,1	338	229	100	9
C	80,0	442	235	193	14
D	82,4	435	245	174	16
E	52,4	294	213	74	7
		L.S.D. = $56\frac{1}{4}$	N.S.	L.S.D. = $25\frac{3}{4}$	–

1 lb = 0.4536 kg.
L.S.D. = *G.D. – l'écart minimum.*
N.S. = *nicht signifikant – non significative.*

TABELLE 5. *Wirkung von Behandlungen auf Auflaufen, Gesamtertrag und Ertrag in Speise- und Saatsortierung sowie an kleinen Knollen (Erträge in lb)*

TABLEAU 5. *Effet des traitements sur la levée, le rendement total et le rendement en commerciaux, plants et grenaille (rendement en lb)*

treatments reduced it significantly, especially the TBTA treatment where the phytotoxic effect was again obvious. Washing alone also adversely affected emergence and moreover, the cold late spring of 1962 probably tended to exaggerate deleterious effects on tubers caused by treatment.

For yield determinations the plants were allowed to come to maturity naturally. Lifting was carried out in mid-October, the tubers from each treatment were weighed, then graded over $2\frac{1}{4}$ in $\times$ $1\frac{1}{4}$ in riddles into ware, seed and chats and the weights of each grade determined. Results are summarised in TABLE 5.

Significant reductions in total yield were given by TBTA treatment and also washing alone – the washing effect involves complex questions which will be discussed in future papers dealing specifically with the mechanisms involved in the washing and disinfection process. It is hardly surprising that yields were so much reduced in both cases, because of the high percentage of blanking and also because many stems of plants which did emerge were weak and slow to develop, particularly in the TBTA treatment. Analysis of the grading results shows much of the loss to be caused by a reduction in the amount of seed-size tubers produced from the TBTA and washing treatments, but it is noteworthy that most seed was produced following dipping in MEMC.

6. CONCLUSION

Although some of the organo-tin compounds like TBTA were quite active in destroying sclerotia of *R. solani* they cannot at present be recommended for use as disinfectants on tubers at lifting time because of their phytotoxicity. It is possible that this could be reduced by suitable formulation as only technically-pure substances along with a wetting agent were used in these experiments. HARTEL (1962) does not believe

phytotoxicity of trialkyltins can be significantly reduced by formulation, but such questions are properly the sphere of the industrial firms.

In the Netherlands and elsewhere in Europe disinfection is, in many instances, carried out much later after lifting than is the case in Scotland, and usually unwashed tubers are used. If organo-tin compounds were employed in these circumstances, a different disease control and phytotoxic picture might well be obtained. After some time in storage, tuber skins are much less susceptible to chemical damage whereas hardened sclerotia are more difficult to kill. If disinfection was delayed until tubers were sprouting, almost total destruction of the sprouts by the trialkyltins would occur. This was observed in a small-scale test carried out with tubers (var. *Up-to-Date*) hand-washed and disinfected with TBTO, TPTO, TPTA and TBTA in late February, 1960, where the tubers were showing tiny sprouts. TBTO and TPTO caused almost 100% death of sprouts, whereas TBTA and TPTA were slightly less toxic. It is noteworthy, however, that the skins of the tubers remained largely unaffected. In such cases of severe sprout damage there would probably be an even poorer emergence and a higher yield reduction than in the two trials mentioned here. Nevertheless, it might be worth trying disinfection of unwashed, unsprouted tubers which have been stored some time before treatment.

As was explained earlier, the phytotoxicity of the very active fungicide DEOTO could not be assessed because an insufficient quantity of the chemical was available. However, its use in commercial practice cannot be considered because it is very difficult to synthesize on a large scale and, since no other use for it has yet been found, the cost would presumably be prohibitive. There seems little prospect of the organotins superceding organo-mercury compounds as potato tuber treatments in Scotland in the near future.

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SUMMARY

Organo-mercury compounds are used on a commercial scale in Scotland for disinfecting potato tubers to control various tuber diseases and tuber-borne pathogens such as *Rhizoctonia solani*, but the compounds are very poisonous to mammals and could present a hazard under certain conditions. Certain organo-tin compounds, some of which show promise as active fungicides, are much less toxic, and a number of these were screened for their killing action on sclerotia of *R. solani*.

Good results were obtained with two substances, one of which (diethyloctyltin oxide) was difficult to synthesize chemically and was therefore too expensive for commercial use. The other (tributyltin acetate) being cheaper and readily available, was compared with an organo-mercury disinfectant (methoxyethylmercury chloride) on a field scale. Both chemicals adequately controlled dry rot (*Fusarium caeruleum*) and gangrene (*Phoma foveata*), but the tin compound had a powerful phytotoxic action on the tubers which result-

ed in considerable loss of treated tubers in store and caused marked reductions in emergence and yield when tubers which survived the treatment were grown on.

It it concluded that, at present, organo-tin compounds are unlikely to supercede organo-mercury compounds for disinfecting potato tubers immediately after lifting in Scotland.

ZUSAMMENFASSUNG

DIE ANWENDUNG VON ORGANISCHEN ZINN-VERBINDUNGEN ALS DESINFEKTIONSMITTEL FÜR KARTOFFELKNOLLEN, BESONDERS GEGEN *Rhizoctonia solani*

In Schottland werden organische Quecksilber-Verbindungen in handelsüblichem Rahmen zur Desinfektion von Kartoffelknollen angewendet, um verschiedene Knollenkrankheiten und knollengebundene Krankheitserreger, wie *Rhizoctonia solani*, zu bekämpfen. Diese Verbindungen sind aber für Säugetiere giftig und könnten unter gewissen Umständen eine Gefahr bedeuten. Die Suche nach weniger giftigen Substanzen ist deshalb weitergeführt worden. Gewisse organische Zinn-Verbindungen, die viel weniger giftig als die quecksilberhaltigen Mittel, aber offenbar wirksame Schädlingsbekämpfungsmittel sind, wurden einer Prüfung unterzogen.

Die Wasserlöslichkeit der organischen Zinn-Verbindungen wurde zuerst bestimmt, da bekannt ist, dass dieser Eigenschaft große Bedeutung in der Wirksamkeit gegen *R. solani* zukommt, siehe Ergebnisse in TABELLE 1. Die Verbindungen wurden dann auf gleiche Weise, wie dies für organische Quecksilber-Verbindungen gilt (GRAHAM, 1960), auf die abtötende oder hemmende Wirkung gegenüber Sklerotien von *R. solani* durchgetestet, indem Lösungen oder Suspensionen angewendet wurden, die 100 p.p.m. Zinn plus ein Netzmittel enthielten. Die Ergebnisse sind in TABELLE 2 zusammengestellt. Sie zeigen, daß zwei Verbindungen, nämlich Tributyl-Zinn-Azetat (TBTA), und Diäthyloctyl-Zinn-Oxid (DEOTO), sehr wirksam sind gegen Sklerotien, doch wurde DEOTO nicht weiter geprüft, da es sehr schwierig war, diese Verbindung chemisch herzustellen und sie deshalb im Handel wahrscheinlich zu teuer wäre.

Ein Problem bei den organischen Zinn-Verbindungen ist ihre Phytotoxizität. Um diese beurteilen zu können, wurde ein kleiner Ertragsversuch durchgeführt, dessen Ergebnisse in TABELLE 3 angegeben sind. Auf Grund der obenerwähnten Versuchsergebnisse wurde TBTA ausgewählt und im Vergleich zu einem organischen Quecksilber-Desinfektionsmittel (Methoxyäthyl-Quecksilberchlorid, MEMC) in einem Feldversuch angewendet, um vor allem die Phytotoxizität, besonders nach mechanischem Waschen, festzustellen. Für diesen Versuch wurden Knollen der Sorte *Majestic* 28 Tage nach dem Abbrennen der Stauden mit schwefliger Säure geerntet und innerhalb 48 Stunden nach der Ernte mit folgenden fünf Verfahren behandelt:

A. Nur in Kisten aufbewahrt.
B. Gewaschen in einer Kartoffelwaschmaschine mit 4,9 kg/cm² (70 lb/in²) Wasserdruck, dann in Kisten eingefüllt.
C. Gewaschen wie unter B, 12 Minuten eingetaucht in MEMC-Lösung, enthaltend 100 p.p.m. Quecksilber + Netzmittel, dann in Kisten eingefüllt.
D. Gewaschen wie unter B, 12 Minuten eingetaucht in MEMC-Lösung, enthaltend 50 p.p.m. Quecksilber + Netzmittel, dann in Kisten eingefüllt.
E. Gewaschen wie unter B, 12 Minuten eingetaucht in TBTA-Lösung, enthaltend 100 p.p.m. Zinn + Netzmittel, dann in Kisten eingefüllt.

Nach der Behandlung wurden die Knollen getrocknet und bis zur Pflanzzeit in Kisten gelagert. Beide Präparate bekämpften die Trockenfäule (*Fusarium caeruleum*) und Phoma-Knollenfäule (*Phoma foveata*) ausreichend, doch verursachte TBTA beträchtliche Schäden an den Knollen (TABELLE 4). Die überlebenden Knollen wurden in einem 5 × 5 Lateinischen Quadrat im Feld ausgepflanzt und hernach Auflaufen und Ertrag bestimmt (TABELLE 5). Die Ergebnisse zeigen, daß TBTA – verglichen mit MEMC – eine merkliche Verschlechterung im Auflaufen und im Ertrag verursachte. Daraus wird geschlossen, daß gegenwärtig organische Zinn-Verbindungen wahrscheinlich kaum die organischen Quecksilber-Verbindungen als Desinfektionsmittel für Kartoffelknollen übertreffen, wenn Knollen unmittelbar nach der Ernte damit behandelt werden, wie es in Schottland in der Praxis üblich ist.

RÉSUMÉ

L'UTILISATION DE COMPOSÉS ORGANIQUES D'ÉTAIN POUR LA DÉSINFECTION DE TUBERCULES DE POMME DE TERRE, PARTICULIÈREMENT CONTRE *Rhizoctonia solani*

Les composés organo-mercuriques sont utilisés sur une échelle commerciale en Ecosse pour maîtriser diverses maladies et agents pathogènes du tubercule, tel que *Rhizoctonia solani;* toutefois ces composés sont toxiques pour les mammifères et peuvent présenter un danger sous certaines conditions. Une recherche de substances moins toxiques a été poursuivie et certains composés organiques d'étain ont été examinés, qui sont beaucoup moins toxiques que les mercuriques tout en étant apparemment des fongicides actifs. En premier lieu la solubilité dans l'eau des composés organiques d'étain a été déterminée parce qu'il est connu que c'est là un important facteur d'activité contre *R. solani;* les résultats sont donnés dans le TABLEAU 1. Les composés sont alors classés suivant leur action fongicide ou inhibitrice sur les sclérotes de *R. solani*, suivant la manière décrite par GRAHAM (1960) pour les composés organo-mercuriques, c'est-à-dire en utilisant des solutions ou suspensions contenant 100 parties par million (p.p.m.) d'étain plus un agent mouillant. Les résultats sont résumés dans le TABLEAU 2. Ils montrent que deux composés, à savoir le tributylacétate d'étain (TBTA) et le diéthyloctyloxide d'étain (DEOTO), sont très actifs contre les sclérotes; toutefois les recherches avec DEOTO, dont la synthèse chimique est difficile et qui, par conséquent, aurait probablement été trop onéreux pour une utilisation commerciale, n'ont pas été poursuivies.

Se pose le problème de la phytotoxicité des composés organiques d'étain; pour établir celle-ci, un petit essai de production fut fait dont les résultats figurent au TABLEAU 3. Sur la base des résultats obtenus, TBTA fut retenu pour être comparé avec un désinfectant organo-mercurique (le méthoxyéthyl chlorure de mercure, MEMC), dans une nouvelle et vaste expérience sur champ, afin de déterminer la phytotoxicité, particulièrement après lavage mécanique. Dans cette expérience les tubercules de la variété *Majestic* sont récoltés 28 jours après brûlage complet des fanes à l'acide sulfurique et subissent les cinq traitements suivants 48 heures après arrachage:

A. mis en caisses sans autre traitement;
B. lavés dans une machine à laver les pommes de terre à 4,9 kg/cm² (70 lb/in²) de pression d'eau, puis mis en caisses;
C. lavés comme en B., trempés 12 minutes dans une solution de MEMC contenant 100 p.p.m. de mercure plus le mouillant, puis mis en caisses;
D. lavés comme en B., trempés 12 minutes dans une solution de MEMC contenant 50 p.p.m. de mercure plus le mouillant, puis mis en caisses;
E. lavés comme en B., trempés 12 minutes dans une solution TBTA contenant 100 p.p.m. d'étain plus le mouillant, puis emmagasinés.

Après traitement, les tubercules sont séchés puis conservés en caisses jusqu'au moment de la plantation.

Les deux produits chimiques maîtrisent suffisamment la pourriture sèche (*Fusarium caeruleum*) et la gangrène (*Phoma foveata*), mais TBTA cause des dommages chimiques considérables aux tubercules (TABLEAU 4). Les tubercules restants sont plantés au champ en parcelles disposées en carré latin; on détermine la levée et le rendement (TABLEAU 5). Les résultats révèlent que TBTA provoque des réductions nettes de levée et de production comparativement à MEMC; l'on conclut que, pour le moment, les composés organiques d'étain sont inefficaces pour remplacer les composés organo-mercuriques comme désinfectants des tubercules de pomme de terre quand on les utilise dans le traitement immédiatement après arrachage, ce qui constitue la pratique habituelle en Ecosse.

REFERENCES

ANON (1958): Stalinon: a therapeutic disaster. *Brit. med. J.* **1**, 515.

BARNES, J. M., and H. B. STONER (1958): Toxic properties of some dialkyl and trialkyltin salts. *Brit. J. industr. Med.* **15**, 15–21.

BOYD, A. E. W. (1960): Fungicidal dipping and other treatments of seed potatoes in Scotland. *Europ. Potato J.* **3**, 137–154.

GRAHAM, D. C. (1960): Control of *Rhizoctonia solani* on potato by disinfection of seed tubers with organo-mercury compounds. *Europ. Potato J.* **3**, 80–89.

———, S. N. S. SRIVASTAVA and C. E. FOISTER (1957): The control of *Rhizoctonia solani* on potato. *Plant Path.* **6**, 149–152.

HARTEL, K. (1958): Organic tin compound as a crop fungicide. Tin and its uses. *Quarterly Journal of the Tin Research Institute.* No. 43, 9–14.

——— (1962): Triphenyltin compounds. A new fungicidal group of active substances – their biological properties and their application to agriculture. *Agric. and Vet. Chemicals.* **3**, 19–24.

HOLMES, T. D., and I. F. STOREY (1962): Comparative trials of copper, dithiocarbamate and organotin fungicides for blight control in Lincolnshire 1958–1961. *Plant Path.* **11**, 139–146.

KERK, G. J. M. VAN DER, and J. G. A. LUIJTEN (1954): Investigations on organo-tin compounds. 3. The biocidal properties of organo-tin compounds. *J. appl. Chem.* **4**, 314–319.

LINT, M. M. DE, and J. H. VAN EMDEN (1957): Control of Black Scurf and Stem Canker (*Rhizoctonia solani* KUHN) by disinfection of seed potatoes. *Prov. IVth International Congress of Crop Protection Hamburg*, 1957. **2**, 1443–1444.

LYLE, W. H. (1958): Lesions of the skin in process workers caused by contact with butyltin compounds. *Brit. J. industr. Med.* **15**, 193–196.

PIETERS, A. J. (1961): Triphenyltin hydroxide, a fungicide for the control of *Phytophthora infestans* on potatoes and some other fungus diseases. *Proc. British Insecticide and Fungicide Conference.* **2**, 461–470.

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TAXONOMY OF THE SOFT ROT COLIFORM BACTERIA

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INTRODUCTION

At the risk of stating the obvious, I must emphasize at the outset that soft rot bacteria are grouped together because they produce soft rot in plant tissue. They induce a special kind of biochemical breakdown of tissue. They do not dessicate or mummify tissue; rather, they make it wet and slimy. In that taxonomic sense, the grouping of these bacteria is the result of similarity within their "enzyme battery."

The state of the taxonomy of the soft rot bacteria is hardly commensurate with their importance to plant pathology, and plant pathologists. Indeed, it is almost as confused as the plant tissues after their attack!

At first I hoped to deal with the taxonomy of all the soft rot organisms, but such full consideration is not possible within the limits of the review. Therefore I shall consider only those coliform organisms which are pathogenic to plants.

The soft rot bacteria to be dealt with here have one morphological and five biochemical characters in common. They are physically alike in being peritrichously flagellated rods and biochemically alike in being pathogenic to plants, gram-negative, facultatively anaerobic, in reducing nitrate to nitrite, and in fermenting many carbohydrates to acids. Given these characters in common, what other properties may be used to produce meaningful groupings of the organisms?

In the postwar era, great strides have been made in the various areas of general bacteriology; that is, in cytology, bacterial biochemistry, serology, bacteriophagy, mode of pathogenesis, genetics, and immunochemistry. All of these factors should be considered if we are to create a firm taxonomic structure. The failure to do this in the case of the coliform plant pathogens has not been overlooked (1), although the situation has improved during the last few years (2).

Soft rot is the symptom that results from the destruction of the cellulose of cell walls or of the pectin substances in the wall matrix or in the middle lamella of plant cells. The cells separate and the protoplast dies. There follows a series of complex chemical and physical reactions, which give rise to the typical syndrome. Research on the effects of soft rotting fungi such as *Botrytis cinerea*, *Penicillium expansum*, and many others has enlarged our understanding of these processes for the soft rotting bacteria.

It is convenient to divide the issues facing us regarding the taxonomy of the soft rot coliform bacteria into (*a*) the question of the family and genus to which the organisms belong; (*b*) the problem of the species and infraspecific taxa which compose that genus; and (*c*) a consideration of newer knowledge of these bacteria in relation to their taxonomy. We will examine

the first topic from an historical aspect and then consider the views as they began to diverge in different countries after the First World War.

THE FAMILY AND GENUS PROBLEM

At the turn of the century a number of workers discovered that certain bacteria induce soft rot diseases in plants. The first definite record appears to be that of soft rot of carrot in the United States by Jones (3), who found it to be caused by a motile, peritrichously flagellated bacillus. Almost simultaneously, van Hall (4) in Holland and Appel (5) in Germany, discovered, quite independently, that an organism of similar morphology causes blackleg disease of potato. Soon soft rots on many kinds of plants were found to be caused by these bacteria. Bacteriologists began classifying the isolated organisms, and referring them to the three popular schemes of classification in use at that time, namely, those of Migula, Lehmann & Neumann, and Erwin F. Smith. In all three classifications the soft rot organisms were placed in the family *Bacteriaceae;* but in Migula's classification they were referred to the genus, *Bacillus,* while in Smith's or Lehmann & Neumann's to the genus *Bacterium.* After the First World War new workers appeared who tended to become more isolated, crystallizing their views on a regional basis. Furthermore, the science of plant pathology was expanding rapidly and a schism began to develop between phytopathologists and bacterial systematists. Burkholder (6) has explained this aspect admirably.

The United States.—In the United States, a committee of the Society of American Bacteriologists (7, 8) decided to establish within the *Bacteriaceae* a new tribe, the *Erwineae,* on the sole basis of plant pathogenicity. Erwin Smith and most subsequent workers thought this to be an unsound practice. Nevertheless, the committee did point out the value of biological as well as morphological characters in the delineation of taxa. Following these suggestions, the first volume of Bergey's *Manual of Determinative Bacteriology,* published in 1923, contained within the tribe *Erwineae,* two genera, *Erwinia* Winslow et al. and *Phytomonas* Bergey. *Erwinia* was restricted to the peritrichously flagellated organisms, whereas *Phytomonas* was erected for the other phytopathogens.

In 1937, Rahn (9) reviewed the characters of the *Eubacteriales* and suggested the introduction of a new family, Enterobacteriaceae, which contained one genus, *Enterobacter,* a name embracing, *inter alia,* the genus, *Erwinia.* This emphasized the relationship of the peritrichously flagellated pathogens on plants to the enteric bacteria in animals, a fact which had been recognized among bacteriologists for a number of years. Certain workers reported, for example, that some rot diseases were caused by *Escherichia coli* and *Aerobacter* spp. (10). The family, Enterobacteriaceae was accepted in the fifth edition of Bergey's *Manual* (1939), but was divided into five tribes largely on biochemical grounds, though the *Erwineae* were still distinguished primarily by their plant pathogenicity. Examination of the sixth (1948) and

seventh (1957) editions of Bergey's *Manual* shows that the position has remained essentially the same.

It has long been recognized that the genus, *Erwinia*, consists of at least two distinct kinds of organisms, morphologically similar, but differing in their pathogenicity, and in their nutritional and biochemical, properties. One group is responsible for dry necroses and wilt diseases. It contains the type species of the genus, *E. amylovora* (Burrill) Winslow et al., the causal organism of pear fire blight. The members of the second group are responsible for the true soft rots because, unlike the first, they secrete pectolytic enzymes. I consider that this is a fundamental and reproducible difference between the groups and Waldee (11) drew attention to it. He suggested that the two groups should constitute separate genera, *Pectobacterium* for the soft rot organisms and *Erwinia* for the nonpectolytic group. He even suggested erecting a new family, the *Erwiniaceae* to contain *Erwinia*, so distinctive did he consider these organisms. The division of the original genus, *Erwinia*, into the two genera (but retaining both in the Enterobacteriaceae) has been accepted in some parts of the world, but among other workers, Burkholder (12) has argued against its acceptance.

Finally, the views of certain medical bacteriologists in the United States should be mentioned. In recent years the so-called "newer" biochemical tests (which will be discussed in a later section) have been applied extensively in characterizing members of the Enterobacteriaceae. Because of the deficiency of knowledge of the reactions of the plant pathogenic coliforms to these tests, Edwards & Ewing (13) oppose placing them in the Enterobacteriaceae. "We need more data," they say.

Great Britain.—Bergey's *Manual* has tended to become internationally accepted as bacteriology's taxonomic *vade mecum*. But this is not the case in Britain, where many experienced bacteriologists have used other methods of classification. The family, *Bacteriaceae*, and the genus, *Bacterium*, were retained for the colon bacterium and its relatives, and with these were placed the peritrichously flagellated plant pathogens. This view was supported by the late W. J. Dowson and other British plant pathologists. Dowson felt that retaining the generic name *Bacterium* grouped together organisms which were closely allied and this arrangement reflected relationships by laying the greatest emphasis on their similarities, whereas the American system tends to draw attention to their differences [for a fuller account see Dowson (14, 15); Wilson & Miles (16)].

When the International Committee on Bacteriological Nomenclature finally decided in 1953 to reject the family *Bacteriaceae* and the genus *Bacterium* (17) and later to conserve the family, Enterobacteriaceae (18), workers in Britain were finally obliged to accept a new nomenclature. Some took over the American system, but in 1957 Dowson (15) formally adopted the Waldee *Erwinia-Pectobacterium* division, although he also included the gall-forming, strictly aerobic *Agrobacterium* species in the redefined genus,

Erwinia. Later, Graham & Dowson (19) reiterated that the Waldee separation is justified. In spite of this, the use of *Pectobacterium* is not yet widely accepted in Britain.

France.—Hauduroy, Ehringer, Urbain, Guillot & Magrou, in their *Dictionnaire de Bactéries Pathogènes*, published in 1937 (20), adopted the classification of Bergey. Later, Magrou & Prévot (21, 22) suggested placing the gram-negative plant pathogens in several genera, including *Erwinia*, in a much modified newly defined *Pseudomonadaceae*. This classification is unrealistic but has been adopted by some French workers and it appears in the second edition of the *Dictionnaire*, published in 1953 (23).

More recently, a somewhat different suggestion with regard to the taxonomy of *Erwinia* has been discussed by Moustardier et al. (24). Their view is that the genus, up to now, has been studied largely by phytopathologists, but that its representatives are also of interest to medical bacteriologists and others because the organisms are widely distributed in nature and can be confused with members of the Enterobacteriaceae. They consider that if *Erwinia* is to be incorporated in the Enterobacteriaceae, the genus should be broken up and its members distributed among the previously recognized groups according to their major biochemical characteristics. It is concluded that *Erwinia* would therefore either disappear or possibly be retained only for the motile gas-forming species. This is an interesting proposition. Hamon & Peron (25) found that certain coliphages would lyse soft rot coliform cultures and this was taken as further evidence that the bacteria belonged to the Enterobacteriaceae.

Soviet Union.—Krasil'nikov (26) has divided peritrichously flagellated plant pathogens into two genera, *Bacterium*, for nonpigmented forms (including, of course, the soft rot bacteria) and *Chromobacterium* for pigmented forms. This division has little merit and, in any case, the pigmented bacteria have little in common with the genus *Chromobacterium* as defined by Sneath (27). Israil'ski (28, 29) and Gorlenko (30) followed Bergey's *Manual* but later Gorlenko & He (31) followed Dowson in using *Pectobacterium*.

Denmark.—Hellmers has accepted the *Erwinia-Pectobacterium* split (32, 33). Kauffmann (34), though primarily interested in human and animal pathogens, also considered plant pathogens. He would place *Erwinia* in the tribe *Klebsielleae* of the *Enterobacteriaceae* together with several other genera, all of which are characterized by capacity to grow in the presence of potassium cyanide and failure to produce glutamic acid decarboxylase. These properties have not been used by plant pathologists and comprise part of the so-called newer biochemical tests.

Yugoslavia.—Těsič conceived the idea of placing in the genus *Bacterium* all the plant pathogenic species discussed in his 1949 monograph (35) and 1953 review (36). On seeing Krasil'nikov's work, he reconsidered and suggested adopting this system (37). Again, he reviewed the situation in 1962 (38), but did not suggest any major changes.

India.—In 1951, Patel & Kulkarni (39) suggested that a separate family, *Phytobacteriaceae,* should be created to contain all the plant pathogens. This is similar in concept to the old tribe, *Erwineae.* It would contain, among other genera, both *Erwinia* and *Pectobacterium;* these two workers recognizing the importance of the biochemical property of degrading pectin.

In other parts of the world, the Bergey classification is adopted to a large extent. Stapp (40), in Germany, Săvulescu (41, 42) and Lazar & Bucur (43) in Rumania, Goto & Okabe in Japan, and numerous other plant pathologists have set the seal of approval on the classification by using it extensively in their writings.

Digest of Family and Generic Relationships of Soft Rot Coliforms

It seems clear that the soft rot peritrichously flagellated bacilli are related to the enteric bacteria and that they may safely find a home in the Enterobacteriaceae. The evidence in favour appears to be overwhelming and anyone handling these organisms over a period of time cannot fail to be struck by their overall similarity to the colon bacillus and its relatives. I believe few bacteriologists would disagree with this view.

More controversial is the problem of the genus, *Erwinia.* A comparison of members of the soft rot group with the fire blight group such as *E. amylovora, E. nigrifluens* Wilson, Starr & Berger (44), and *E. tracheiphila* (Smith) Holland reveals striking differences in many properties. Burkholder (12) hesitated, but enough knowledge has accumulated on the properties of both groups of organisms for systematists to examine the question again. For instance, much work has been done in Britain on *E. amylovora* since the discovery of fire blight in southeast England in 1957 (45). We cannot discuss all the data here, but Table I summarizes some differences between soft rot coliforms and the fire blight organism.

Having studied several cultures of the above three species I agree with Waldee (11), that the organisms are unlike members of the Enterobacteriaceae. In fact, they seem more like certain members of the *Achromobacteriaceae* and some workers feel that they might be accommodated here. Burkholder (46) suggests a resemblance between *E. tracheiphila* and an organism isolated from the gut of the cucumber beetle and which was referred to *Achromobacter superficiale* by Steinhaus (47). But transferring the organisms to the *Achromobacteriaceae* only adds to the confusion because the family is a very ill-defined collection of peritrichously flagellated and nonmotile rods which do not fit well into other families. Perhaps, as Waldee suggests the family, *Erwiniaceae,* could be resurrected to accommodate them. The yellow pigmented plant saprophytic bacteria referred to as *E. lathyri* (Manns & Taubenhaus) Holland might find a place here, too. This species is no longer described in Bergey's *Manual,* 1957 edition, presumably because of its doubtful taxonomic position. It was relegated to the abortive *Index Bergey-*

TABLE I

SOME DIFFERENCES BETWEEN *E. amylovora* AND SOFT ROT COLIFORMS

Test	*E. amylovora*	Soft Rot Coliform
Gas from glucose	−	d
Acid from glycerol	+	d
Acid from lactose	−	+ or (+)
Acid from raffinose	d	+
Acid from inulin	−	+
Acid from dulcitol	−	d
Acid from xylose	V	d
Acid from rhamnose	V	+
Acid from maltose	V	d
Acid from cellobiose	(+)	+
Acid from salicin	(+)	+
Utilization of citrate	+	d
Utilization of malonate	−	d
Production of indole	−	d
Methyl red	−	d
Voges-Proskauer	−	d
Production of H_2S	−	d
Reduction of nitrate	−	+
Gluconate test	+ or V	−
Liquefaction of pectate gel	−	+
Growth requirement for nicotinic acid	+	−
Lysis by *E. amylovora* phage	+	−
Lysis by *E. atroseptica* phage	−	d
Host range	Restricted	Wide

Notation for biochemical tests: + = positive 1–2 days; (+) = delayed positive; V = variable results in different experiments; d = different isolates give consistently different reactions. The tests for acid production from carbohydrates were carried out in an inorganic basal medium plus bromothymol blue indicator. Data are from various sources.

ana. I examined some of the biochemical properties of *E. lathyri* (48) and gathered that it is closer to the *E. amylovora* group than to the soft rot coliforms. At the same time, Hawaiian cultures of the pineapple pathogen, *E. ananas* Serrano, were found to be very similar if not identical with *E. lathyri;* even the absorption spectra of the xanthophyll pigments are alike. In this paper I suggested that other reportedly phytopathogenic yellow-pigmented species such as *E. mangiferae* (Doidge) Bergey et al., *E. citri-maculans* (Doidge) Magrou, and *E. cassavae* (Hansford) Burkholder might be merely strains of *E. lathyri*, and the various diseases with which they are associated should be re-examined with this in mind. Certainly, cassava leaf spot is now known to be caused by *Xanthomonas cassava* Wiehe &

Dowson (49) and not by *E. cassavae*. Further evidence for the interrelation of *E. amylovora* with *E. lathyri* and *E. ananas* was provided by studies with *E. amylovora* phage by Billing et al. (50); the phage lysed *E. lathyri* and *E. ananas*, but not soft rot coliforms. Other organisms which may belong here are certain yellow chromogens which agricultural bacteriologists have usually referred to the *coli-aerogenes* group (51). More recently, it has become recognized that plant surfaces commonly carry organisms which may best be described as nonpigmented saprophytic *Erwinia* spp. (52).

There remain at least two organisms in the present genus, *Erwinia*, which, in my opinion, do not fit into either *Pectobacterium* or the *E. amylovora* group. These are *E. nimipressuralis* Carter, associated with wet wood disease of elm (53), and *E. carnegieana* Lightle, Standring and Brown, the causal agent of a disease of the giant cactus, *Carnegiea gigantea*. A thorough reinvestigation of the cactus disease was made in the United States by Boyle (54), who considered the organism to be closely related to *E. carotovora*.

On the other hand, I examined supposedly authentic cultures of both organisms and found that they do not produce pectolysis, and, hence, cannot be soft rot coliforms. Their physiological and biochemical properties follow those of the *Cloaca* group, i.e., *Aerobacter* (*Enterobacter*) *cloacae*, even to the extent of oxidizing gluconate to 2-ketogluconate and decarboxylating lysine, which soft rot coliforms cannot do. In this connection it is interesting to find that an organism identified as *E. nimipressuralis* was isolated from sausages in Algiers by Brisou, Tysset & Jacob (55). Brisou and his colleagues seem to find *Erwinia* like bacteria in many places, including trout [the organism was considered a new species, *E. salmonis* (56)], and in the estuary of the Charente River (57). From the description, the isolate from the sausages seems to be a soft rot coliform as it is said to rot potato slices rapidly, and gives the biochemical reactions of the group. Probably it originated from the dirty water which the authors say is sometimes used to adulterate the sausages.

I conclude that these observations indicate that ample evidence can be adduced to justify the separation of *Erwinia, sensu stricto* and *Pectobacterium*. This does not reduce *Erwinia* to one or two isolated organisms but to the nucleus of a group of both saprophytic and phytopathogenic bacteria which could form a coherent taxon.

THE SPECIES AND INFRASPECIFIC TAXA

Speciation in the soft rot coliform group is in a state of utter taxonomic confusion. As Starr (2) states "This is an inevitable concomitant of the general practice, when reporting a previously undescribed disease, of presenting an inadequate description of the "new" species with usually no, or only feeble, attempts at direct bacteriological comparison with possible related species. What is even more deplorable—and much less understandable—are the equally feeble attempts to explore rigorously the host range of the new species." He continues with the comment that the usual emphasis lies in

proving the uniqueness of the new species by merely reporting its isolation from a unique host. This attitude is summed up in the little anecdote about the old naturalist who found a sparrow in a pear tree and called the bird *Passer pyri*!

While agreeing with this criticism, I think it should not be forgotten that large numbers of isolates of soft rot coliforms from many parts of the world have not been generally available for comparative study until fairly recently. However, since the establishment of the National Collection of Plant Pathogenic Bacteria at Harpenden, England, the situation has eased considerably.

Another practice adds to the confusion. Often the discoverer of a "new" culture compares its biochemical properties with only one or two so-called typical isolates of the known and already established species. However, it is a matter of debate whether typical cultures of the established species exist. Therefore, to say that "the organism differs in ten important characters from the other species" can be dangerously misleading. The experienced microbiologist, having handled many isolates representing all the species, realizes that differences between isolates included in the same species are almost always as great as those shown between species.

Notwithstanding these difficulties, some of the older problems of the identity of organisms from different hosts in different parts of the world have been cleared up successfully. This progress is reflected in the fact that of 18 specific epithets of characteristic soft rot coliforms contained in Elliott's *Manual* (58), only three of these remain in the latest Bergey's *Manual*, namely, *Erwinia carotovora* (Jones) Holland, *E. atroseptica* (van Hall) Jennison, and *E. aroideae* (Townsend) Holland. Since Elliott's publication, a further species, *E. chrysanthemi*, erected by Burkholder, McFadden & Dimock (59) has appeared in Bergey's *Manual* and, subsequent to its publication, another species has been described by McFadden, namely, *E. dieffenbachiae*, the cause of a stem and leaf rot of *Dieffenbachia* spp. (60). Together with these species is an assorted collection of varieties and *formae speciales* (f. sp.) which have been designated by various investigators. Before going on to a discussion of these bacteria, however, we should examine several poorly defined coliform organisms which are associated with soft rot.

Ill-defined soft rot bacteria.—Erwinia cytolytica Chester (61), is the name given to an organism isolated from stem rot of dahlia in the United States. Although a somewhat similar disease has recently been discovered in Rumania [Lazar, (62)], and a culture of the causal organism deposited in the National Collection at Harpenden, it is as yet impossible to say whether the organism is a very distinctive soft rot coliform. *E. dissolvens* (Rosen) Burkholder, originally obtained from maize in 1921 (63) and which was considered to be an *Aerobacter* by Waldee (11), may be a case of mistaken identity. A sugar cane pathogen from Argentina and one said to cause a root rot of cotton in the Soviet Union are mentioned in the literature, although,

like *E. cytolytica*, they have disappeared from the seventh edition of Bergey. These bacteria are respectively, *E. flavidad* (Fawcett) Magrou and *E. erivanensis* (Kalantarian) Bergey et al. They seem to be yellow pigmented strains of soft rot coliforms. In fact, yellow organisms have been obtained from other sources, though rarely. Graham (48) isolated one from potato and Dowson had another in his culture collection, though the host was not stated. Biochemically, these two are indistinguishable from the coliform group.

E. rhapontici (Millard) Burkholder, the cause of crown rot disease of rhubarb in the north of England (64), was studied by Metcalfe (65). It seems to be a true coliform, although it produces a pink pigment which distinguishes it from other members of the soft rot group. It also differs in that it has a restricted host range. It rots cucumber and onion slices, but not slices of potato tuber, carrot, or turnip. Since the organism has not been found in recent years, information on its characteristics is out of date. Although a culture is in the National Collection, it is impossible to be certain whether or not its properties have altered significantly during the time lapse before it was preserved by freeze drying. A decision on the taxonomic relationships of this pathogen must await the rediscovery of the disease and isolation of fresh cultures.

Speciation in the Commonly Known Soft Rot Coliforms

As was pointed out by Graham & Dowson (19), "ever since Jones (3) described *Bacillus carotovorus* as the cause of soft rot of carrot, van Hall (4) *B. atrosepticus* as the cause of potato blackleg and Townsend (66) *B. aroideae* as the cause of soft rot of arum lily, workers have disputed whether these three should be designated as different species or variants of a single species." Graham & Dowson continue "The basis for separating the soft rot coliform bacteria into species, variants, and *formae speciales* has always been controversial. It has rested largely on characteristics of fermentative ability and pathogenicity, while little attention has been paid to serology or the newer biochemical tests now widely used by medical bacteriologists such as growh in cyanide media or the production of amino acid decarboxylases." With these observations in mind, their pathogenic properties and biochemical activities will be examined in turn.

Pathogenicity.—Economically speaking, blackleg of potato is probably the most important disease caused by soft rot coliforms. This is not surprising, considering the widespread cultivation of the potato both as food and a source of carbohydrate for chemical processing. Much work has been done on the blackleg organism in attempting to distinguish it from similar bacteria found on other hosts, and Hellmers (33), in a review of nomenclatural problems relating to the blackleg organism, mentions all relevant papers. Some early investigators found that potato isolates, when inoculated into potato stems, readily produce blackleg while the majority of isolates from

other hosts do not. Assorted biochemical differences were also detected, and on these criteria some workers felt that the blackleg organism should be separated from the other soft rot coliforms and given specific rank. Considerable weight was afforded to this view by the extensive work of Burkholder & Smith (67) and Smith (68) who maintained that there are two distinct species, *E. atroseptica* (the blackleg organism) and *E. carotovora*. Burkholder & Smith by that time did not recognize the existence of *E. aroideae*, an organism generally regarded as distinct from *E. carotovora* because it fails to produce gas from sugars. Later, Holdeman & Burkholder (69) isolated an organism identified as *E. aroideae* from tobacco. In India, Hingorani & Addy (70) reached essentially the same conclusions—they recognized two species, *E. carotovora* and *E. atroseptica*, and considered *E. aroideae* as a nongasforming strain of *E. carotovora*.

But other investigators, notably Rudd Jones (71) and Hellmers & Dowson (72), were able to produce blackleg disease with all soft rot coliform isolates. Experiments by the latter two workers demonstrated the importance of introducing the organisms directly into the vascular tissue of the stems to obtain infection and, using this method, they produced blackleg with an isolate which was reported to be nonpathogenic towards potato stems by Burkholder & Smith.

In our early work at this station, using Burkholder & Smith's procedure (67), we, too, thought that at least two kinds of organisms are involved (73). We resolved this perplexing problem when we found (19) that temperature is as vital a factor in disease production as is the site of introduction of inoculum. All of 25 isolates, which originated from different hosts in different countries, can cause blackleg symptoms when the inoculated plants are held at 76° F (24.5° C) or above, while some can also produce blackleg at 66° F (19° C) or below. Those which belong to the so-called high temperature group largely originated in tropical or subtropical countries or from plants cultivated in hothouses outside the tropics and, *inter alia*, includes *E. carotovora* and *E. aroideae*. The low temperature group, almost exclusively derived from temperate regions, includes the organism most frequently found in cases of natural blackleg infection, namely, *E. atroseptica*.

Also included in our potato pathogenicity tests were the organisms causing slow wilt of carnation (32, 74, 75), bacterial blight of chrysanthemum (59), the leaf rot disease of *Philodendron* (76), and bacterial disease of *Parthenium* (guayule) caused by an organism named *E. carotovora f. sp. parthenii* by Starr (77). These belong to the high temperature group, producing blackleg like symptoms above 70° F, and all were referred to *E. chrysanthemi*.

Taking a closer look at the diseases which these organisms produce on their natural hosts, we find that, particularly in the case of carnation wilt and to a less extent, blight of chrysanthemums and the guayule disease, the syndrome is fundamentally that of a true wilt and not of soft rot. Indeed

one of the diagnostic features of carnation wilt is the masses of bacteria embedded in a gelatinous matrix that block the conducting tissues of the stem. Here is something different, and we naturally ask ourselves the question "will these organisms cross infect in their various hosts?" The tests which have been carried out have given equivocal results. For example, the carnation isolates will cause chrysanthemum blight but the chrysanthemum organisms apparently, will not cause carnation wilt (32), whereas the chrysanthemum and *Parthenium* organisms will infect certain species of *Philodendron* (76). What most workers do seem to agree on, however, is that isolates corresponding to *E. carotovora*, *E. aroideae*, and *E. atroseptica* can never attack any of these hosts which, incidentally, are plants grown in nurseries so that opportunities for cross infection may arise from time to time. Also in the *chrysanthemi* group may belong *E. dieffenbachiae*, an organism which I have not studied. It was originally described by Munnecke (78), who considered it to be a strain of *E. chrysanthemi*. Though he was unable to produce an active infection on chrysanthemum with it, he drew attention to its property of spreading in the xylem vessels (and in the resin ducts) of Diefenbachia in a way similar to that reported for the organisms of carnation wilt and chrysanthemum blight. McFadden (60), who investigated a similar disease in Florida, decided, on the basis of host range and biochemical tests, to give the organism specific status. The disease was also recently found in West Germany (79).

Bacterial rots of maize occur in many parts of the world, and Sabet (80) studied the disease as it occurs in Egypt. He concluded that at least one organism responsible is a soft rot coliform, but in cross-inoculations he found that *E. atroseptica*, *E. carotovora*, and *E. aroideae* isolates are unable to attack maize. The bacterium is similar in other respects to *E. carotovora* and since the strain is adapted to infect maize, he proposed that it should be given the name, *E. carotovora f. sp. zeae*. Later, Sabet (81) examined an organism from rot of maize in Southern Rhodesia and concluded that it is very similar to the Egyptian pathogen. At the same time, he suggested that the organism found attacking maize and sorghum in Australia (82) is probably identical with the African isolates. Since then, another bacterium, also considered to correspond with *f. sp. zeae*, has been found on maize in India (83). In the United States Boewe (84) ascribed a stalk rot of maize in Illinois to *E. carotovora*. Kelman, Person & Herbert (85) found a similar disease in North Carolina, but did not specifically name the organism, though they remarked that it differed from other species both biochemically and in its pathogenicity to maize. Volcani, working in Israel, found a bacterial stalk rot for the first time in 1957 (86), but the organism isolated was identified as *E. aroideae* and was not thought to be the primary cause of the disease. Volcani (87) noted a similar disease again in 1959, but a somewhat different bacterium was isolated from affected specimens. She compared it with the Southern Rhodesian *f. sp. zeae*, and concluded that the two were identical

although the results of certain biochemical tests were different from those recorded by Sabet (80), and the organism was designated only as *E. carotovora*.

An interesting disease of sugar cane called bacterial mottle was found in Queensland by Steindl (88). The symptoms consist of chlorotic striping of the leaves which become entirely invaded as are also the sheaths and stems, causing severe stunting, wilting, and death. The organism responsible, which was said to be very like the potato blackleg pathogen (89), was found infecting several grasses in the same area and was successfully inoculated into sorghum and maize. In these hosts it produced leaf mottles and some plants developed a rot; it was named *Pectobacterium carotovorum* var. *graminarum* by Dowson & Hayward, who studied its biochemical properties (90). In cross-inoculation studies, I found both this and the Southern Rhodesian maize pathogen will produce blackleg in potato at 76° F and above.

Finally, the soft rot coliform which causes a pineapple disease in Malaya should be mentioned. Apparently first noted in 1935, both a heart rot of the young plants and a soft rot of the fruits can be produced (91). Attempts to inoculate the hearts of pineapple plants with isolates of *E. carotovora*, *E. atroseptica*, and *E. aroideae* proved to be negative and Dowson (92) suggested it should be designated a *forma specialis—f. sp. ananas*.

Biochemical properties.—Phytopathologists interested in soft rot coliforms carry out certain biochemical tests, which form part of the standard diagnostic procedures for these organisms. Diagnosis is fairly easily established because experience has shown that the chances are that any motile gram-negative, nonpigmented organism which liquefies pectate gel and produces acid from salicin in an inorganic basal medium, is a member of this group [if it is yellow-pigmented it might be a coliform or a *Flavobacterium*, so care is needed (93)]. Biochemical differences have long been sought which would distinguish between organisms with different pathogenic characteristics and which would also identify organisms from soil or other general habitats. It is commonly considered that pathogenicity differences might be reflected in the biochemical properties; the number of papers on this theme published over the last 60 years is ample confirmation of the widespread nature of this belief.

A great deal of work has centered on trying to distinguish the potato blackleg organisms from other soft rot pathogens, and one of the earliest differences noted was that the blackleg organism is aerogenic whereas some isolates from hosts other than potato are anaerogenic. The first anaerogenic bacterium studied was Townsend's arum lily (*Calla*) pathogen and, since that time, failure to produce gas from sugars has become the hallmark of *aroideae*, irrespective of whether or not the organism could attack arum lily [in this context it is noteworthy that some isolates from lilies have been found which produce gas (94)].

The production of acid from maltose is a property which numerous

workers have attempted to use to characterize the blackleg organism. For example, Dowson (95) stated that *E. carotovora* did not ferment maltose, whereas *E. atroseptica* did so with the production of acid and gas, but it is now generally recognized that this is probably wrong, for organisms are known which ferment maltose, but which cannot be considered as *E. atroseptica* for other reasons (96).

A somewhat similar situation is found with regard to the formation of acid from ethanol. Originally ethanol was added to media in varying concentrations to discover the alcohol tolerance of the organisms (97), but later Massey (98) found some of them metabolize the substance, and he introduced ethanol agar as a medium to distinguish between *E. aroideae* and *E. atroseptica*, both of which produce no change in the medium, and *E. carotovora*, which causes a marked fall in pH. Since that time, alcohol has been incorporated in many kinds of basal media and there have been several confirmations and denials that the ethanol reaction is of diagnostic value (67, 72, 99). Dowson (15) stated that both *E. carotovora* and *E. aroideae* produce acid on ethanol agar, whereas *E. atroseptica* does not, but Graham & Dowson (96), while agreeing that the statement is generally true, showed that certain organisms, which could not be considered as *E. atroseptica*, do not form acid on the medium. In further experiments, we found that cultures which produced acid on the agar also formed acid in 5 per cent ethanol peptone broth, but the ethanol beef extract peptone broth recommended by Burkholder & Smith (67) as a differential medium, produced variable results. Using the microtest method (100, 101) with washed suspensions grown on nutrient agar and incubated in 5 per cent buffered ethanol, it was demonstrated that acid production in the ethanol alone is perfectly correlated with its production in ethanol agar and ethanol peptone broth. This indicates that the acid is not necessarily formed from other substances present in these relatively complex media.

Nothing has been more contradictory than the reports of the methyl red (M.R.) and Voges-Proskauer (V.-P.) reactions, probably because different media and times of incubation were used. For instance, it was reported by Burkholder & Smith (67) that the reactions of *E. carotovora* and *E. atroseptica* were M.R. positive, V.-P. negative in ordinary glucose phosphate peptone, but, on the other hand, Rudd Jones (71) stated that all strains of soft rot coliforms were M.R. negative, V.-P. positive in O'Meara's (102) fumarate broth. Taylor (103) tested the M.R. and V.-P. reactions of 18 isolates, comparing ordinary glucose phosphate peptone medium with O'Meara's fumarate medium and also the medium of Smith, Gordon & Clark (104). His observations showed that the Smith, Gordon & Clark medium is useless for coliforms. Of the fumarate medium, he remarked that, since it gave many double positives and negatives, it should not be employed exclusively, otherwise a valuable differential criterion could be lost. Work in this laboratory with over 50 isolates has confirmed Taylor's

views; many give double positives with the fumarate, and this number is increased if Barritt's (105) modification to detect traces of acetoin (acetylmethylcarbinol) with naphthol is used. With ordinary glucose phosphate peptone, Graham & Dowson (96) reported that *E. atroseptica* was M.R. positive, V.-P. negative, while the majority of the other cultures were M.R. negative, V.-P. positive. Eddy (106) reviewed the significance of the V.-P. reaction. In relation to its taxonomic and diagnostic value, he concludes "the important taxonomic character is the ability of the bacteria to carry out the reactions leading to the formation of acetoin." Since acetoin is likely to be further metabolized to 2,3-butanediol and other products, it is suggested that the most sensitive methods available should be used to perform the V.-P. test to confirm the presence of the enzyme system responsible for acetoin production. On this basis, most soft rot coliforms would be recorded as V.-P. positive.

The capacity of members of the Enterobacteriaceae to produce acid from lactose has been considered to have considerable diagnostic value, and it may be argued that some bacteriologists have overstressed its significance. For example, the genus, *Paracolobactrum* Borman et al. was established on this criterion, and though few workers have accepted this proposal, it still appears in the 1957 edition of Bergey's *Manual*. Most isolates of soft rot coliforms produce acid from lactose quickly, but some are late or irregularly positive. These include the organisms from chrysanthemum blight, carnation wilt, and the diseases of *Philodendron*, *Dieffenbachia*, and *Parthenium*. The organisms from maize in Egypt and Southern Rhodesia were also reported by Sabet (80, 81) to give a delayed lactose fermentation, but in our laboratory they gave acid within two days and it is noteworthy that Volcani (87) confirmed this.

The majority of soft rot coliforms give a negative test for formation of indole (or methylindole) from tryptophan, but there are a number of examples of these organisms which produce indole. Quite often we find that there are discrepancies in the results recorded by different workers using the same organism. For instance, Graham & Dowson (96) stated that the chrysanthemum blight bacterium is indole-positive, whereas Miller & McFadden (76), like Burkholder, McFadden & Dimock (59), reported it as negative or only a trace. Here we have an illustration of subjective differences in interpretation of results. It is quite true that the indole reaction appears to be much weaker in this case than, say, with the *Philodendron* bacterium which gives a strong rose pink colouration with Kovacs' *p*-dimethylaminobenzaldehyde reagent, but the colour given by the chrysanthemum isolates can be intensified somewhat by the addition of 0.1 per cent tryptophan to the medium. In the opinion of this reviewer, the important taxonomic character is whether or not the bacterium possesses the enzyme stystem necessary to convert tryptophan to indole (or methylindole) and not the extent to which indole may be found in the medium at any given time. Another possible

cause for discrepancies in results may be due to differences in methods of testing. Indole is relatively volatile and gives a positive reaction with Gnezda oxalic acid test papers suspended above the medium, but if methylindole is formed instead, no colouration is given as the substance is less volatile (74).

Other information concerning biochemical properties is referred to in the papers already quoted, such as acid production in dulcitol, raffinose, utilization of malonate, hippurate, and many others, but space precludes their consideration. They have not been as widely used as the reactions already discussed and consequently their comparative value remains doubtful.

Digest of Specific and Infraspecific Divisions of Soft Rot Coliforms

Taking stock of the data which has formed the basis for the establishment of species and subspecific divisions, what do we find? The organisms designated *aroideae* have been consistently characterized by a single biochemical property, namely, failure to produce gas from carbohydrates. The difficulty is that this property cannot be satisfactorily related to others. Furthermore, it has long been known that the gas production by freshly isolated strains is unstable and can be soon lost in culture so that they could then be wrongly identified as *aroideae*. To say the least, it seems unwise to establish a species on one criterion and this is why some workers give such organisms only varietal status (15, 72, 96). Nevertheless, this property is particularly helpful in the diagnosis of freshly isolated strains, and must not be entirely ignored for taxonomic purposes.

The organisms which give a delayed lactose fermentation seem to have better possibilities of forming a separate taxon than the nongasformers. These bacteria are not only capable of producing soft rots but also true wilts, typified by carnation slow wilt. In this context, it is noteworthy that Hellmers (32), considering that all these organisms exhibit a basic similarity, raised the infrasubspecific name *f. sp. parthenii* to specific rank on grounds of priority and made the chrysanthemum and carnation pathogens varieties of this species (var. *chrysanthemi* and var. *dianthicola*). As Burkholder (12) pointed out, this classification cannot be accepted, as it does not accord with the rules of nomenclature and therefore *chrysanthemi* must remain the legitimate name. Hellmers also discusses the possibility of dividing the genus into two groups called *stirps*, namely, *stirps carotovora* and *stirps parthenii* which are distinguished biochemically. He suggests that a type species be designated within each *stirps* and the rest of the deviating isolates of the *stirps* are then characterized as varieties of the type species. The *stirps* concept is reminiscent of the taxon, *subgenus*, and it is doubtful if its use has any real value in helping to clarify the taxonomy of these bacteria. Cross-inoculation tests have so far failed to confirm that members of the delayed lactose-fermenting group are pathogenically identical, but it should be remembered that there are pitfalls in interpreting too sweepingly the implica-

tions of results of host range tests. When inoculations have failed, it cannot be tacitly assumed that infections could never be established under any other conditions. Perhaps the culture was too old; or unsuitable inoculation techniques were used; or the plants were not in a susceptible physiological state; or the wrong horticultural varieties were employed; or the environment of the host was unsuitable during the incubation period. The blackleg controversy is an example of the difficulties that host range tests can cause. With these possibilities in mind, we might speculate that, for example, the organism causing stem rot of *Dieffenbachia* might infect some varieties of chrysanthemum under suitable conditions.

This kind of criticism can be levelled at the use of the term, *forma specialis*. Recommendation 8a of the Bacteriological Code (107) defines the *forma specialis* as "A subdivision of a species of a parasitic or symbiotic microorganism distinguished primarily by adaptation to a particular host." The maize pathogen was designated *f. sp. zeae* because it was believed to be adapted to disease production on *Zea*, but it is impossible to be sure that all organisms from other hosts and with different characteristics are therefore necessarily unable to attack this plant. In fact, the organism from sugar cane mottle will attack maize, although perhaps this is not a good example as cultures seemed to behave more like the maize isolates than the blackleg pathogen. The only way to be certain that an organism will attack a given host is to test it under a variety of conditions. For obvious practical reasons, it is impossible for workers to carry out tests to determine the complete host range characteristics of every isolate, steps which would be essential to identify specifically an organism from soil or other general habitat. This point is often reiterated in the literature, and while its significance must be clear to plant pathologists, it usually goes unheeded, or merely has lip service paid to it (12, 96, 108).

But the question mark against the use of *forma specialis* in the context of the maize organism does not end there. Although the Bacteriological Code states that the *f. sp.* is a parasite or symbiote adapted to a particular host, an annotation draws attention to the fact that the concept of the *f. sp.* is derived largely from mycology, where it has proved useful, *inter alia*, in characterizing plant rusts. Some *Puccinia* species contain forms so specialized that they will attack wheat and barley but not oats or rye; others from oats will attack rye or wheat, and yet others from rye will not cause disease on wheat or barley. It is these which are given the status of *formae speciales* and from this example we see that adaptation to a host also implies a restriction of host range. But as far as can be judged, the maize organism does not show host range restriction concomitantly with adaptation to its host, for tests show it can produce soft rots on all kinds of fleshy vegetables and fruits and will attack tobacco, tomato, and potato stems.

If the words "distinguished primarily by adaption to a particular host" are taken at their face value, then a number of so called species could be

named as *formae speciales*, as was indeed done by Gorlenko & He (31), who designated *f. sp. typicum*, *phytophthorum* (i.e., *atrosepticum*), *aroideae*, *melonis*, and *zeae*. This argument applies particularly to the pathogen associated with potato blackleg, which is an organism that finds the environment and methods of cultivation and propagation of the potato particularly suitable for its own perpetuation. But the blackleg pathogen is capable of attacking many other hosts. For instance, it has been found on tomato, celery, and iris under field conditions in Scotland. What appears to be the blackleg organism also causes a disease of larkspur in the United States (109) and there the pathogen is perpetuated through seed borne infection.

An alternative to the use of the taxon, *forma specialis*, for such organisms is to recognize them as ecotypes, a suggestion already put forward by Crosse & Garrett (110) in connection with the taxonomy of the phytopathogenic pseudomonads. Another way of looking at the question is to argue that by planting crops man, in effect, "cultivates" pathogens (111) and the use of the taxon, *cultivar*, in the sense used in the International Code for Cultivated Plants (112) might be appropriate. But the acid test is whether it is useful terminology and this is open to question, for it would be confusing especially when the organisms are found on hosts other than those from which they are usually isolated.

When it comes to attempting to characterize the various pathogens by biochemical tests, a stalemate is reached because the tests which have so far been employed are inadequate. All that the tests can do is to give an indication of potential pathogenic reactions. Graham & Dowson (96) put forward a simple scheme where one species, *Pectobacterium carotovorum*, was divided into four—*carotovorum* itself and the varieties *aroideae*, *atrosepticum*, and *chrysanthemi* [later wrongly altered to *parthenii* by Dowson, Graham & Hellmers (113), who believed this name to have precedence over *chrysanthemi*]—on the basis of eight biochemical reactions and pathogenicity towards potato stems at high and low temperatures. This scheme, which leaves *carotovorum* for all organisms not fitting into the varietal categories, would not satisfy the taxonomic purist, but is helpful for the plant pathologist, and in the meantime there seems to be no good reason for changing it.

BIOCHEMICAL TESTS

This and the following two sections give some idea of the recent work which is helpful in further elucidating the relationships of the soft rot coliforms.

Many biochemical tests have been developed during the last 25 years which have considerable value in bacterial taxonomy. One of the most important was the introduction by Moeller (114) of simple tests for the production of amino acid decarboxylases and the arginine dihydrolase system. Numerous observations have confirmed the importance of these tests in studying members of the Enterobacteriaceae (115, 116). The production of

phenylpyruvic acid by deamination of phenylalanine (117, 118) is another such reaction, particularly helpful in distinguishing the *Proteus* group. The gluconate test, which depends on the oxidation of gluconate to reducing substances such as 2-ketogluconate, was originally used by Haynes (119) for the characterization of pseudomonads and has since been used for Enterobacteriaceae (120). The presence of oxidase in bacterial cells is easily determined by a spot test using the tetramethyl-*p*-phenylenediamine dihydrochloride reagent impregnated on filter paper, a method developed by Kovacs (121). Again it was first used for distinguishing certain pseudomonads, but since then it has proved to have wider applications. Tests for the production of urease and for growth in the presence of potassium cyanide are also usually employed today.

Very little has been published on the biochemical reactions of soft rot coliforms as shown by these tests, but investigations on these lines have been continuing in our laboratory over the last few years. Parallel with newer tests, the older tests for fermentation of sugars, nitrate reduction, etc., have been carried out. It is impossible for lack of space to detail the exact methods used in all the experiments, but some explanation is necessary. Decarboxylases were tested for in several ways and the most consistent results were given by Moeller's (114) method, whereas equivocal results were given by the methods devised by Carlquist (122) and Falkow (123) and by microtests (115). As is pointed out by Edwards & Ewing (13), with the Moeller method the brand of peptone used is undoubtedly critical—Orthana Meat USPXV must be used. The presence of the decarboxylation products in fluid from microtests was checked by paper chromatography and cultures of other Enterobacteriaceae with known decarboxylase properties were run simultaneously with the soft rot bacteria, as controls.

Production of phenylalanine deaminase was detected by microtests (115) and with phenylalanine agar (124); urease with Christensen's (125) urea agar. The gluconate test was performed according to Graham & Dowson (96); growth in KCN using Moeller's (13) medium; and presence of oxidase detected by Steel's (126) modification of the Kovacs (121) technique. For fermentation tests, the medium consisted of peptone water plus bromothymol blue indicator plus 1 per cent w/v of sugar, and final readings were made after incubation for 14 days at 26° C. Utilization of citrate was determined by growth in Koser's citrate medium (127) after seven days incubation, and utilization of malonate determined, using a modified Leifson malonate broth (128). The methods for testing for production of hydrogen sulphide and indole, reduction of nitrate, gelatin liquefaction, and liquefaction of pectate gel were those given in Graham & Dowson (96) except that the tryptone water for the indole test had 0.1 per cent tryptophan added to it. For methyl red and Voges-Proskauer reactions, organisms were grown in ordinary glucose phosphate peptone for five days at 26° C. The culture was then divided into three, one portion being tested with methyl red indicator, one portion

for the presence of acetoin by the caustic potash-creatine method, and another for acetoin by Batty-Smith's (129) modification of Barritt's (105) method.

Altogether, 57 cultures isolated in 12 different countries were used in the experiments. They were obtained from the following host plants (the figure that follows the host is the number of isolates from that host): potato 20, maize 2, celery 2, avocado pear 2, iris 2, tobacco 2, tomato 4, *Calla* (arum) lily 2, carrot 2, Chinese cabbage 2, chrysanthemum 2, pepper (*Capsicum*)

TABLE II

BIOCHEMICAL REACTIONS OF 57 STRAINS OF SOFT ROT COLIFORMS

Biochemical test	Numbers		Biochemical test	Numbers	
	+	−		+	−
Motility	55	2	Methyl red	37	20
Gas from glucose	34	23	Voges-Proskauer	23	34
Acid from glucose	57	0	Voges-Proskauer (Barritts)	47	10
Acid from mannitol	56	1	Urease	0	57
Acid from dulcitol	0	57	Nitrate reduction	57	0
Acid from inositol	12	45	Gluconate test	0	57
Acid from sorbitol	0	57	Lysine decarboxylase	1	56
Acid from lactose	51	6[a]	Ornithine decarboxylase	3	54
Acid from maltose	26	31	Glutamic acid decarboxylase	0	57
Acid from rhamnose	56	1	Arginine dihydrolase	3	54
Acid from sucrose	57	0	Oxidase test	0	57
Acid from inulin	53	4	Malonate test	22	35
Acid from salicin	57	0	Growth in KCN	42	15
Acid from ethanol	14	43	Growth in Kosers citrate	55	2
Indole produced	7	50	Phenylalanine deaminase	0	57
H_2S produced	7	50	Pectate gel liquefaction	57	0
Gelatin liquefaction	57	0			

[a] Late or irregularly positive or negative.

2, onion 1, radish 1, cabbage 1, carnation 2, poppy (*P. somniferum*) 1, *Parthenium* 1, pineapple 1, *Philodendron* 1, sugar cane 1, *Schizanthus* 1, *Sauromatum* 1; one was isolated directly from field soil in Scotland. This collection was chosen to give as wide a geographical distribution and host range as possible and contains cultures received as *E. atroseptica*, *E. carotovora*, *E. aroideae*, *E. chrysanthemi*, *E. carotovora f. sp. parthenii*, *E. carotovora f. sp. zeae*, *P. carotovorum* var. *graminarum*, as well as the pathogens of carnation wilt, *Philodendron* rot and pineapple heart rot. About another 200 isolates from potato in Scotland were available but were excluded so that

excessive weighting of the data with information on organisms restricted to one host in one geographic area could be avoided.

The first and rather striking fact that can be learned from the table is the remarkable uniformity in the reactions to certain newer tests such as production of decarboxylases and deaminase, despite the diverse origins of the organisms. It necessarily follows that these tests will not help to distinguish between the different pathogenic types of bacteria, and confirms the opinion of Graham & Dowson (96) that they do not facilitate delineation of species. These results do show, however, that soft rot coliforms comprise a biochemically well defined group close to the *Cloaca* group and possibly to the *Hafnia* group of the medical bacteriologist, but differ by not forming certain amino acid decarboxylases and also by giving a negative gluconate test. [Compare results with biochemical characteristics of other groups of the Enterobacteriaceae given in Tables included in (1, 13, 130).]

A coliform which causes a respiratory infection in man known as rhinoscleroma and which is usually referred to as *Klebsiella rhinoscleromatis* Trevisan resembles the soft rot coliforms in not possessing amino acid decarboxylases and in giving a negative gluconate test, but it differs by being nonmotile, unable to liquefy gelatin and by failing to utilize citrate as a sole source of carbon. Seven isolates tested in our laboratory did not cause pectolysis on potato tuber slices when incubated at 26° C. In summation, we may say that the results favour the designation of a *Pectobacterium* genus within the Enterobacteriaceae.

SEROLOGY

Since the beginnings of serology, many investigators, with infinite labour, have gradually unravelled the complex antigenic structure of coliform bacteria, culminating in the monumental Kauffmann-White schema for the diagnosis of *Salmonellae*. Compared with this detailed knowledge, the information on the serology of the soft rot coliforms is fragmentary.

Early work demonstrated the serological heterogeneity of bacteria from different hosts though those from the same host showed some similarity (131, 132, 133). But Stapp (134) was the first to compare a large number of isolates. He divided these isolates into five serotypes which, over the years, were increased to nine (40, 135, 136). On very tenuous grounds the serotypes were designated as *formae speciales* of a single species termed, *E. phytophthora*, namely, *f. sp. solani*, *brandenburgensis*, *carotovorae*, *iridis*, *hyacinthi*, *brassicae*, *betae*, and *taraxaci*.

Elrod (137) noted the correlation between serological specificity and maltose fermentation. He concluded that the common antigenic components of the soft rot group resided in the flagella, and absorption experiments revealed a large number of components. The somatic antigens were believed to be primarily type-specific, though there was some evidence of common somatic factors. Work by Mushin, Naylor & Lahovary (138) with somatic

antisera confirmed that different isolates showed marked antigenic variability and cross-agglutinations.

By far the most exhaustive investigations so far have been conducted in Japan by Goto & Okabe (139–143). Flagellar antigen assays on 180 isolates from different hosts in different localities indicated that they could be divided into 12 serological groups labelled A-L and the groups subdivided into a further 70 strains. The association of certain antigens in the bacteria with capacity to attack maltose was again observed, and some correlation of pathogenicity with serotype was detected. Flagellar antigens were shown to be monophasic in the majority of serotypes, and using a nonflagellated isolate, the typical OH variation was found to occur. Cross-agglutination experiments with somatic antisera suggested the presence of group-specific and strain-specific somatic antigens in all isolates and also the occurrence of capular (K) antigens, which were thermolabile.

Because of this great heterogeneity in antigenic structure, Starr (2) questioned the validity of Nováková's (144) results. The latter found that only those isolates from diseased potatoes which were capable of rotting potato slices gave slide agglutinations with antiserum prepared against the potato blackleg organism. Our observations (145) have, however, confirmed Nováková's work; it just happens that the blackleg bacterium is more or less antigenically homogeneous.

Serology of the phytopathogens generally has not evolved to the extent it has done in the medical sphere, and in this imperfect state, its value is in making quick diagnoses. As further knowledge accumulates, the place of serology in elucidating taxonomic relationships will become clearer.

ROTTING ENZYMES

Soft rot bacteria possess enzymes which degrade pectic substances. Partly because of the association of pectolytic enzymes with plant pathogenicity, there has been much investigation of pectin breakdown brought about by both bacteria and fungi. The subject has been reviewed extensively by Starr (2, 146), Husain & Kelman (147), and Wood (148). What one gathers is that the pectolytic enzymes of soft rot bacteria form a complex, the resolution of which is made difficult both by lack of knowledge on the precise chemical nature of the substrates (149, 150) and the exact stucture of the middle lamella itself (151).

So far, pectin methylesterase seems the one enzyme best correlated with pathogenicity (152). More recently, investigations have been carried out on pectin transeliminase (153), but it remains uncertain whether pectolytic enzymes are also toxins capable of killing protoplasts or whether other nonenzymic substances are involved. So far, analysis of this enzyme complex has not helped to clarify the taxonomy of the group.

Cellulose degrading enzymes in bacteria are also receiving more attention as it gradually becomes clearer that they may take a significant part

in pathogenic processes. Ammann (154) found that water soluble methylcellulose is attacked by enzymes present in filtrates of *E. aroideae* cultures. Goto & Okabe (155, 156) reported that the ability to liquefy sodium carboxymethylcellulose gels was possessed by many phytopathogenic bacteria, but I could not demonstrate this in over 50 isolates of the potato blackleg organism. Gehring (157) made an extensive study of carnation slow wilt disease, finding that soft rot coliforms from arum lily, iris, potato, pear, and swede were unable to infect carnation. Analysis of their pectolytic and cellulolytic properties compared with the slow wilt pathogen indicated that, in general, the carnation organism was less active pectolytically and more active cellulolytically than the other bacteria. Gehring suggested that differences in power to cause a wilt and multiply in the xylem as distinct from a soft rot reflects differences in the precise nature of the enzymes secreted by the organisms. These conclusions support Wood's (148) contention that cellulases are more important in slowly developing diseases where the pathogen is associated with the host for relatively long periods.

FURTHER DEVELOPMENTS

Much general information relating to the soft rot coliforms has had to be omitted from this review, particularly regarding metabolic studies, though this is covered by Starr (2) to 1959. This field is perhaps not of immediate interest to plant pathologists, but comparative biochemistry is assuming more and more importance in taxonomy as the enzymic make up of different families becomes clearer. An excellent general picture is given by De Ley (158). From these painstaking analytical studies, simplified tests for the presence of enzymes can be evolved, enabling bacteriologists to use them regularly for routine diagnostic and determinative purposes. A recent example is the easy detection of β-galactosidase and β-galactoside permease, enzymes concerned with lactose utilization, developed by Le Minor & Ben Hamida (159).

Some fresh thinking on the mechanism of pathogenicity might bring us valuable data linking it with biochemical properties. The answer may not lie in pectolytic enzymes but in the production of other substances, not necessarily toxins, which are as yet undetected. Paton (160), working with pathogenic pseudomonads, demonstrated death of plant cells caused by a diffusible substance which was not a pectinase. Pretreatment of tissues, such as potato tuber, with the powerful calcium and magnesium chelating agent, sodium ethyldiaminetetraacetic acid (EDTA), greatly increased the rate of cell disintegration. Moreover, Paton found it possible to isolate pseudomonads from the soil which, although possessing polygalacturonase activity, were unable to rot susceptible tissue unless it was treated with EDTA. This seems to point to a chelation reaction as a primary step in pathogenesis, and chelating substances could be looked for among the metabolic products of the soft rot coliforms.

Changes are also taking place in the methodology of taxonomy, especially since the introduction of numerical taxonomy into bacteriology by Sneath (161, 162). Taxonomic groups are constructions of the taxonomist but the "natural" taxa are formed operationally by placing together organisms with an overall similarity. The process is subjective and therefore biased, but use of computers to analyze and compare data, based on Adansonian principles, may be considered as objective. A description of the various steps in an analysis is given by Sneath (163), and it is clear that the method has merit. So far, it has been applied only briefly to some plant pathogenic pseudomonads and xanthomonads (164). In the meantime, these bacteria offer plenty of scope for further research, the results of which may profoundly affect our views 20 years from now.

CONCLUSION

Admitting freely that not every bacteriologist is in agreement on the taxonomy of the soft rot coliform bacteria, it is my considered opinion that these organisms must be placed in the family Enterobacteriaceae. Their properties are in complete accord with the characters of the family as defined by the Subcommittee on Taxonomy of the Enterobacteriaceae (130).

I am also of the opinion that there is sufficient evidence to justify removing these organisms from the genus, *Erwinia* Winslow et al., and placing them in the genus *Pectobacterium* Waldee, retaining *Erwinia* for the non-pectolytic organisms. I cannot judge whether *Erwinia* should remain in the Enterobacteriaceae. This decision rests with workers who are familiar with these bacteria.

The resolution of the species question is more difficult. But, as I mentioned earlier, I believe that the scheme put forward by Dowson and myself (96) is the best answer at present and I therefore recognize only one species, *P. carotovorum*. This species is divided into four—*P. carotovorum* itself (or, more strictly, *P. carotovorum* var. *carotovorum*), *P. carotovorum* var. *atrosepticum*, *P. carotovorum* var. *aroideae*, and *P. carotovorum* var. *chrysanthemi*.

The chrysanthemum bacterial blight organism, the guayule bacterium, the organism causing leaf rot of *Philodendron*, and the carnation slow wilt organism are all included in var. *chrysanthemi*. Although I have not studied the *Dieffenbachia* stem and leaf rot organism, its properties indicate that it should also be placed in var. *chrysanthemi*. However, it is somewhat doubtful whether the *chrysanthemi* organisms should have only varietal rank. They cause wilt symptoms on certain hosts and, coupled with this, is their property of giving a delayed lactose fermentation. It appears from Gehring's work (157) that these bacteria may also differ in being active cellulolytically. If this fact is substantiated when a large number of isolates have been compared, I would then be in favour of raising var. *chrysanthemi* to specific rank.

For the reasons explained earlier, I consider that the taxon, *forma specialis*, should not be used in designating soft rot coliforms. I therefore do

not accept the use of *f. sp. zeae* to distinguish organisms which cause rots of maize, or *f. sp. ananas* for organisms causing pineapple heart rot. On the same grounds, the sugar cane mottle bacterium should not be given varietal status, because the only character by which this organism supposedly differs from other soft rot coliforms is its capacity to attack certain grasses. In my opinion, all the above three organisms must be absorbed into *P. carotovorum*.

In conclusion, I must emphasize I am well aware that these personal views are open to question. But if this essay does no more than stimulate constructive criticism, it will have gone some way towards solving the taxonomic problems posed by the soft rot coliforms.

LITERATURE CITED

1. Cowan, S. T., Taxonomic rank of Enterobacteriaceae 'groups', *J. Gen. Microbiol.*, **15**, 345–58 (1956)
2. Starr, M. P., Bacteria as plant pathogens, *Ann. Rev. Microbiol.*, **13**, 211–38 (1959)
3. Jones, L. R., A soft rot of carrot and other vegetables caused by *Bacillus carotovorus* Jones, *Vermont Agr. Expt. Sta. Rept.*, **13**, 299–332 (1901)
4. van Hall, C. J. J., *Bijdragen tot de Kennis der Bakteriecle Plantenziekten* (Inaug. dissertation, Univ. Amsterdam, 198 pp., 1902)
5. Appel, O., Der Erreger der "Schwarzbeinigkeit" ber dei Kartoffeln, *Ber. Deut. Botan. Ges.*, **20**, 128–29 (1902)
6. Burkholder, W. H., The genus *Phytomonas*, *Phytopathology*, **20**, 1–23 (1930)
7. Comm. Characterization Classification Bacterial Types, Soc. of Am. Bacteriologists (C. E. A. Winslow, Chmn.), The families and genera of the bacteria, *J. Bacteriol.*, **2**, 560–66 (1917)
8. Comm. Characterization Classification Bacterial Types, Soc. of Am. Bacteriologists (C. E. A. Winslow, Chmn.), The families and genera of the bacteria, *J. Bacteriol.*, **5**, 191–229 (1920)
9. Rahn, O., New principles for the classification of bacteria, *Zentr. Bakteriol. Parasitenk., Abt. II*, **96**, 273–86 (1937)
10. Burkholder, W. H., The taxonomy and nomenclature of the phytopathogenic bacteria, *Phytopathology*, **29**, 128–36 (1939)
11. Waldee, E. L., Comparative studies of some peritrichous phytopathogenic bacteria, *Iowa State Coll. J. Sci.*, **19**, 435–84 (1945)
12. Burkholder, W. H., Present day problems pertaining to the nomenclature and taxonomy of the phytopathogenic bacteria, In *Omagiu lui Traian Savulescu*, 119–27. (Edit. Acad. Republicii Populare, Romine, 1959)
13. Edwards, P. R., and Ewing, W. H., *Identification of Enterobacteriaceae*, (Burgess Publ. Co., Minneapolis, Minn., 258 pp., 1962)
14. Dowson, W. J., *Manual of Bacterial Plant Diseases*, 1st ed. (A. and C. Black, London, 183 pp., 1949)
15. Dowson, W. J., *Plant Diseases due to Bacteria*, 2nd ed. (Cambridge Univ. Press, Cambridge, England, 232 pp., 1957)
16. Wilson, G. S., and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity*, 4th ed. (Edward Arnold, Ltd., London, 2 vols. 2331 pp., 1955)
17. Intern. Comm. Bacteriol. Nom. Minutes of meeting, September, 1953, *Intern. Bull. Bacteriol. Nom. Taxon.*, **3**, 155–61 (1953)
18. Intern. Comm. Bacteriol. Nom. Minutes of meeting, July–August, 1958. *Intern. Bull. Bacteriol. Nom. Taxon.*, **8**, 145–49 (1958)
19. Graham, D. C., and Dowson, W. J., The coliform bacteria associated with potato black-leg and other soft rots, I, Their pathogenicity in relation to temperature. *Ann. Appl. Biol.*, **48**, 51–57 (1960)
20. Hauduroy, P., Ehringer, G., Urbain, A., Guillot, G., and Magrou, J., *Dictionnaire des Bactéries Pathogènes*, 1st ed. (Masson et Cie, Paris, 597 pp., 1937)
21. Magrou, J., and Prévot, A. R., Étude taxonomique des bactéries phytopathogènes, *Compt. Rend.*, **226**, 1229–30 (1948)
22. Magrou, J., and Prévot, A. R., Études de systematique bacterienne, IX, Essai de classification des bacteries phytopathogènes et espéces voisines, *Ann. Inst. Pasteur*, **75**, 99–108 (1948)
23. Hauduroy, P., *Dictionnaire des Bactéries Pathogènes*, 2nd ed. (Masson et Cie, Paris, 692 pp., 1953)
24. Moustardier, G., Brisou, J., Saout, J., and Ehrhardt, J. P., Les Erwinia—discussion taxinomique—interet medical, *Bull. Assoc. Diplomes Microbiol.* Fac. Pharm. Nancy, **82**, 3–12 (1961)
25. Hamon, Y., and Peron, Y., Les propropriétés antagonistes réciproques parmi les Erwinia, Discussion de la position taxonomique de ce genre, *Compt. Rend.*, **253**, 913–15 (1961)
26. Krasil'nikov, N. A., *Opredeletel' bacterii i aktinomitsetov* (Izd-vo Akad. Nauk S.S.R., Moskva, 829 pp., 1949)
27. Sneath, P. H. A., Cultural and biochemical characteristics of the genus *Chromobacterium*, *J. Gen. Microbiol.*, **15**, 70–98 (1956)
28. Israil'ski, V. P., *Bakterielle Pflanzen-*

krankheiten (Deutscher Bauernverlag, Berlin, 375 pp., 1955)
29. Israil'ski, V. P., *Bacterial Diseases of Plants* (In Russian) (Sel'khozgiz, Moskva, 468 pp., 1960)
30. Gorlenko, M. V., Criterion of the species in phytopathogenic bacteria, *Omagiu lui Traian Savulescu*, 39, 259–64 (1959)
31. Gorlenko, M. V., and He, L. Y., Biokhimicheskie svoistva vozbuditelei myagkikh gnilei rastenii Pectobacterium phytophthorum (Appel) Waldee P. carotovorum (Jones) Waldee, P. aroideae (Towns) Waldee [Vestn. Moskov. Univ.] *Ser. Biol.*, 16, 26–36 (1961)
32. Hellmers, E., Four wilt diseases of perpetual-flowering carnations in Denmark. *Dansk Botan. Arkiv*, 18 No. 2, 1–200 (1958)
33. Hellmers, E., *Pectobacterium carotovorum* var. *atrosepticum* (van Hall) Dowson, the correct name of the potato black leg pathogen; a historical and critical review, *European Potato J.* 2, 251–71 (1959)
34. Kauffmann, F., On the principles of classification and nomenclature of Enterobacteriaceae, *Intern. Bull. Bacteriol. Nom. Taxon.*, 9, 1–6 (1959)
35. Těsić, Ž. P., *Fitopatogene Bacterije* (Izdavácko Preduzeće narodne republike srbije, Beograd, 184 pp., 1949)
36. Těsić, Ž. P., Osvrt na noviju nomenklaturu kod fitopathogenih bakterija, *Zastita Bilija*, 18, 68–79 (1953)
37. Těsić, Ž. P., Fitopatogene bakterije u klasifikaciji bakterija N. A. Krasiljnikova, *Zastita Bilija*, 32, 87–92 (1955)
38. Těsić, Ž. P., *Arhiv. Poljopr. Nauk*, 15, 138 (1962)
39. Patel, M. K., and Kulkarni, Y. S., Nomenclature of bacterial plant pathogens, *Indian Phytopathol.*, 4, 74–84 (1951)
40. Stapp, C., *Pflanzenpathogene Bakterien* (Paul Parey, Berlin and Hamburg, 567 pp., 1956)
41. Săvulescu, T., *Analele Acad. Rep. Populare Romane, Ser. III*, 22, 1 (1947)
42. Săvulescu, T., Contribution to the classification of bacterial plant pathogens, *Intern. Congr. Microbiol., 4th, Copenhagen, 1947, Rept. Proc.*, 429–31 (1949)
43. Lazar, I., and Bucur, E., *European Potato J.* (In press)
44. Wilson, E. E., Starr, M. P., and Berger, J. A., Bark canker, a bacterial disease of the Persian walnut tree, *Phytopathology*, 47, 669–73 (1957)
45. Crosse, J. E., Bennett, M., and Garrett, C. M. E., Fireblight of pear in England, *Nature*, 182, 1530 (1958)
46. Burkholder, W. H., Some observations on *Erwinia tracheiphila*, the causal agent of the cucurbit wilt, *Phytopathology*, 50, 179–80 (1960)
47. Steinhaus, E. A., A study of the bacteria associated with thirty species of insects, *J. Bacteriol.*, 42, 757–90 (1941)
48. Graham, D. C., The status of *Erwinia lathyri* and related bacteria, *Commonwealth Phytopathol. News*, 4, 49–51 (1958)
49. Wiehe, P. O., and Dowson, W. J., A bacterial disease of cassava (Manihot utilissima) in Nyasaland, *Empire J. Exptl. Agr.* 21, 141–43 (1953)
50. Billing, E., Baker, L. A. E., Crosse, J. E., and Garrett, C. M. E., Characteristics of English isolates of *Erwinia amylovora* (Burrill) Winslow et al, *J. Appl. Bacteriol.*, 24, 195–211 (1961)
51. Thomas, S. B., and Elson, K., Pigmented strains of *coli-aerogenes* bacteria, *J. Appl. Bacteriol.*, 20, 50–52 (1957)
52. Billing, E., and Baker, L. A. E., Characteristics of *Erwinia-like* organisms found in plant material, *J. Appl. Bacteriol.*, 26, 58–65 (1963)
53. Carter, J. C., Wetwood of elms, *Illinois Nat. Hist. Surv. Bull.*, 23, 407–48 (1945)
54. Boyle, A. M., Further studies of the bacterial necrosis of the giant cactus, *Phytopathology*, 39, 1029–52 (1949)
55. Brisou, J., Tysset, C., and Jacob, A., Recherches sur un germe de la famille des Pseudomonadae (tribu des Pseudomonadeae) Erwinia nimipressularis isolé d'un produit frais de charcuterie, *Arch. Inst. Pasteur Algér*, 38, 181–87 (1960)
56. Brisou, J., Tysset C., and Vacher, B., Etude d'une souche d'Erwinia (*Erwinia salmonis* nv. sp.) isolee d'une truite commune (salmo

fario 1.) *Ann. Inst. Pasteur*, **97**, 241–44 (1959)
57. Brisou, J., Menantaud, J., and Doublet, M., Contribution á l'etude de la flore microbienne normale des estuaires, Les Pseudomonadaceae des boues de la charente, *Bull. Inst. Oceanog.* [Monaco], **1129**, 1–8 (1958)
58. Elliott, C., *Manual of Bacterial Plant Pathogens*, 2nd ed. (Chronica Botanica Co. Waltham, Mass., 186 pp., 1951)
59. Burkholder, W. H., McFadden, L. A. and Dimock, A. W., A bacterial blight of chrysanthemums, *Phytopathology*, **43**, 522–26 (1953)
60. McFadden, L. A., Bacterial stem and leaf rot of Dieffenbachia in Florida, *Phytopathology*, **51**, 663–68 (1961)
61. Chester, F. D., A bacteriosis of dahlia, *Erwinia cytolytica*, *Phytopathology*, **28**, 427–32 (1938)
62. Lazar, I. (Personal communication)
63. Rosen, H. R., Further observations on a bacterial root and stalk rot of field corn, *Phytopathology*, **11**, 74–79 (1921)
64. Millard, W. A., *Publ. Univ. Leeds Yorkshire Coun. Agr. Educ. No. 134*, 1 (1924)
65. Metcalfe, G., *Bacterium rhaponticum* (Millard) Dowson, a cause of crown-rot disease of rhubarb, *Ann. Appl. Biol.*, **27**, 502–8 (1940)
66. Townsend, C. O., A soft rot of calla lily, *U.S.D.A., B.P.I. Bull., No. 60*, 47 pp. (1904)
67. Burkholder, W. H., and Smith, W. L., *Erwinia atroseptica* (van Hall) Jennison and *Erwinia carotovora* (Jones) Holland, *Phytopathology*, **39**, 887–97 (1949)
68. Smith, W. L., Pathogenic differences manifested by *Erwinia atroseptica* and *Erwinia carotovora*, *Phytopathology*, **40**, 1011–17 (1950)
69. Holdeman, Q. L., and Burkholder, W. H., The identity of barn rots of flue-cured tobacco in South Carolina, *Phytopathology*, **46**, 69–72 (1956)
70. Hingorani, M. K., and Addy, S. K., A comparative study of *Erwinia carotovora*, *Erwinia aroideae*, and *Erwinia atroseptica*, *Indian Phytopathol.*, **5**, 40–43 (1953)
71. Rudd Jones, D., On the nomenclature and identity of the coliform soft rot bacteria, *Trans. Brit. Mycol. Soc.*, **33**, 73–81 (1950)
72. Hellmers, E., and Dowson, W. J., Further investigations of potato black leg, *Acta Agr. Scand.*, **3**, 103–12 (1953)
73. Noble, M., and Marshall, M., A note on black leg of potato, *Plant Pathol.*, **1**, 134 (1952)
74. Lelliott, R. A., Slow wilt of carnations caused by a species of *Erwinia*, *Plant Pathology*, **5**, 19–23 (1956)
75. Bakker, M., Bacterieverwelkingszkiekten van Anjers. *Tjdschr. Plantenziekten*, **63**, 20–21 (1957)
76. Miller, H. N., and McFadden, L. A., A bacterial disease of Philodendron, *Phytopathology*, **51**, 826–31 (1961)
77. Starr, M. P., The causal agent of bacterial root and stem disease of guayule, *Phytopathology*, **37**, 291–300 (1947)
78. Munnecke, D. E., Bacterial stem rot of Dieffenbachia, *Phytopathology*, **50**, 696–700 (1960)
79. Sauthoff, W., and Bortels, H., *Gartenwelt*, **62**, 68 (1962)
80. Sabet, K. A., A new bacterial disease of maize in Egypt, *Empire J. Exptl. Agr.*, **22**, 65–67 (1954)
81. Sabet, K. A., A note on the identity of a bacterium causing stalk rot and leaf blight of maize in Southern Rhodesia, *Empire J. Exptl. Agr.*, **25**, 165–66 (1957)
82. Ludbrook, W. U., Top rot of maize, sweet corn, and sorghum, *J. Coun. Sci. Ind. Res. Australia*, **15**, 213–16 (1942)
83. Hingorani, M. K., Grant, U. J., and Singh, N. J., *Erwinia carotovora* f. sp. zeae, a destructive pathogen of maize of India, *Indian Phytopathol.*, **12**, 151–57 (1959)
84. Boewe, G. H., Bacterial stalk rot of corn in Illinois, *Plant Disease Reptr.*, **33**, 342–43 (1949)
85. Kelman, A., Person, L. H., and Hebert, T. T., A bacterial stalk rot of irrigated corn in North Carolina, *Plant Disease Reptr.*, **41**, 798–802 (1957)
86. Volcani, A., A strain of *Erwinia carotovora* isolated from rotten maize plants, *J. Agr. Res. Sta., Rehovot, Ktavim*, **8**, 217–19 (1958)
87. Volcani, Z., A maize stalk disease caused by a strain of *Erwinia carotovora* in Israel, *J. Agr. Res. Sta., Rehovot, Ktavim*, **11**, 179–83 (1961)
88. Steindl, D. R. L., "Bacterial mottle," a new disease of sugar cane in Queensland, *Intern. Sugar J.*, **59**, 174–75 (1957)
89. *Bur. Sugar Expt. Sta. (Brisbane), 58th Ann. Rept.*, 89–90 (1958)

90. Dowson, W. J., and Hayward, A. C., The bacterial mottle pathogen of Queensland sugar cane, *Intern. Sugar J.*, 62, 275 (1960)
91. Johnston, A., Pineapple fruit collapse, *Malay. Agr. J.*, 40, 253–63 (1957)
92. Dowson, W. J. (Private communication)
93. Dorey, M. J., Some properties of a pectolytic soil *Flavobacterium*, *J. Gen. Microbiol.*, 20, 91–104 (1959)
94. Ark, P. A., Starr, M. P., and Sutton, D. D., Bacterial chalk rot of yellow and pink callas, *Phytopathology*, 42, 320–22 (1952)
95. Dowson, W. J., The identification of the bacteria commonly causing soft rot in plants, *Ann. Appl. Biol.*, 28, 102–06 (1941)
96. Graham, D. C., and Dowson, W. J., The coliform bacteria associated with potato black-leg and other soft rots, II, Biochemical characteristics of low- and high-temperature strains, *Ann. Appl. Biol.*, 48, 58–64 (1960)
97. Smith, E. F., *Bacterial Diseases of Plants* (W. B. Saunders, Philadelphia and London, 688 pp., 1920)
98. Massey, A. B., A study of *Bacillus aroideae*, Townsend, the cause of a soft rot of tomato, and *B. carotovorus* Jones, *Phytopathology*, 14, 460–77 (1924)
99. Malcolmson, J. F., A study of *Erwinia* isolates obtained from soft rots and black-leg of potatoes, *Trans. Brit. Mycol. Soc.*, 42, 261–69 (1959)
100. Clarke, P. H., and Cowan, S. T., Biochemical methods for bacteriology, *J. Gen. Microbiol.*, 6, 187–97 (1952)
101. Cowan, S. T., Fermentations: biochemical micromethods for bacteriology, *J. Gen. Microbiol.*, 8, 391–96 (1953)
102. O'Meara, R. A. Q., A simple, delicate and rapid method of detecting the formation of acetylmethylcarbinol by bacteria-fermenting carbohydrates, *J. Pathol. Bacteriol.*, 34, 401 (1931)
103. Taylor, C. B., The soft-rot bacteria of the *coli-aerogenes* group, *Proc. Soc. Appl. Bacteriol.*, 14, 95–100 (1951)
104. Smith, N. R., Gordon, R. E., and Clark, F. E., Aerobic mesophilic sporeforming bacteria, *U. S. D. A. Misc. Publ.*, No. 559, 112 pp. (1946)
105. Barritt, M. M., The intensification of the Voges-Proskauer reaction by the addition of alpha naphthol, *J. Pathol. Bacteriol.*, 42, 441 (1936)
106. Eddy, B. P., The Voges-Proskauer reaction and its significance: a review, *J. Appl. Bacteriol.*, 24, 27–41 (1961)
107. *International Code of Nomenclature of Bacteria and Viruses*, (Iowa State College Press, Ames, Iowa, 186 pp., 1958)
108. Rahn, O., Contributions to the classification of bacteria, *Zentr. Bakteriol. Parasitenk.*, Abst. II, 78, 1–21 (1929)
109. Burkholder, W. H., The causal agents of the black stem disease of annual larkspur, *Plant Disease Reptr.*, 43, 934–35 (1959)
110. Crosse, J. E., and Garrett, C. M. E., Studies on the bacteriophagy of *Pseudomonas mors-prunorum*, Ps. *syringae* and related organisms, *J. Appl. Bacteriol.*, 26, 159–77 (1963)
111. Snyder, W. C., Hansen, H. N., and Oswald, J. W., Cultivars of the fungus, *Fusarium*, *J. Madras Univ.*, 27, 185–92 (1957)
112. *International Code of Nomenclature of Cultivated plants* (Kemink en Zoon, N. V., Utrecht, Netherlands, 28 pp., 1958)
113. Dowson, W. J., Graham, D. C., and Hellmers, E., Naming of some strains of soft-rot coliform bacteria—a correction, *Ann. Appl. Biol.*, 49, 210 (1961)
114. Moeller, V., Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system, *Acta Path. Microbiol. Scand.*, 36, 158–72 (1955)
115. Shaw, C., and Clarke, P. H., Biochemical classification of *Proteus* and Providence cultures, *J. Gen. Microbiol.*, 13, 155–61 (1955)
116. Ewing, W. H., Davis, B. R., and Edwards, P. R., The decarboxylase reactions of Enterobacteriaceae and their value in taxonomy, *Publ. Health Lab.*, 18, 77–83 (1960)
117. Henriksen, S. D., and Closs, K., The production of phenylpyruvic acid by bacteria, *Acta Pathol. Microbiol. Scand.*, 15, 101–13 (1938)
118. Henriksen, S. D., A comparison of the phenylpyruvic acid reaction and the urease test in the differentiation of *Proteus* from other enteric organisms, *J. Bacteriol.*, 60, 225–31 (1950)
119. Haynes, W. C., *Pseudomonas aeru-*

ginosa—its characterization and identification, *J. Gen. Microbiol.*, **5**, 939–50 (1951)

120. Cowan, S. T., Biochemical tests for bacterial characterization, 2, Gluconate test, *Intern. Bull. Bacteriol. Nom. Taxon*, **5**, 141–49 (1955)
121. Kovacs, N., Identification of *Pseudomonas pyocyanea* by the oxidase reaction, *Nature*, **178**, 703 (1956)
122. Carlquist, P. R., A biochemical test for separating paracolon groups, *J. Bacteriol.*, **71**, 339–41 (1956)
123. Falkow, S., Activity of lysine decarboxylase as an aid in the identification of Salmonellae and Shigellae, *Am. J. Clin. Pathol.*, **29**, 598–600 (1958)
124. Ewing, W. H., Davis, B. R., and Reavis, R. W., Phenylalanine and malonate media and their use in enteric bacteriology, *Publ. Health Lab.*, **15**, 153–66 (1957)
125. Christensen, W. B., Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from Salmonella and Shigella types, *J. Bacteriol.*, **52**, 461–66 (1946)
126. Steel, K. J., The oxidase reaction as a taxonomic tool, *J. Gen. Microbiol.*, **25**, 297–306 (1961)
127. Koser, S. A., Utilization of the salts of organic acids by the *coli-aerogenes* group, *J. Bacteriol.*, **8**, 493–520 (1923)
128. Ewing, W. H., Enterobacteriaceae—Biochemical Methods for Group Differentiation, *U. S. Public Health Service Publ. No. 374* (1960)
129. Batty-Smith, C. G., The detection of acetylmethyl-carbinol in bacterial cultures, *J. Hyg.*, **41**, 521–29 (1941)
130. Carpenter, K. P., Report of the subcommittee on taxonomy of the Enterobacteriaceae, *Intern. Bull. Bacteriol. Nom. Taxon.*, **13**, 69–93 (1963)
131. Lacey, M. S., Studies in bacteriosis, XIII. A soft rot of potato tubers due to *Bacillus carotovorus* and a comparison of the cultural, pathological, and serological behavior of various organisms causing soft rots, *Ann. Appl. Biol.*, **13**, 1–11 (1926)
132. Matsumoto, T., On the diagnosis of certain plant infectious diseases by means of serological reactions. *J. Soc. Trop. Agr., Formosa*, **1**, 14–22 (1929)
133. Matsumoto, T., Further studies on some putrefactive phytopathogenic bacteria by agglutinin absorption, *J. Soc. Trop. Agr., Formosa*, **2**, 16–25 (1930)
134. Stapp, C., Die Schwarzbeinigkeit und Knollennassfäule der Kartoffel, *Ab. Biol. Reichanst. Land u. Forst.*, **16**, 643–703 (1928)
135. Stapp, C. Bakterielle Rubenfaulen, *Zentr. Bakteriol. Parasitensk., Abt. II*, **106**, 419–26 (1944)
136. Stapp, C., Bakterielle Erkrankungen der Kautschulspflanze Taraxacum koksaghyz, *Phytopathol. Z.*, **15**, 73–78 (1948)
137. Elrod, R. P., Serological studies of the Erwineae, II. Soft rot group; with some biological considerations, *Botan. Gaz.*, **103**, 266–79 (1941)
138. Mushin, R., Naylor, J., and Lahovary, N., Studies on plant pathogenic bacteria, II. Serology. *Australian J. Biol. Sci.*, **12**, 233–46 (1959)
139. Okabe, N., and Goto, M., Bacterial plant diseases in Japan, II. Studies on soft rots due to *Erwinia aroideae* (Townsend) Holland with special reference to the antigenic structures of flagella, *Bull. Fac. Agr. Shizuoka Univ.*, **5**, 72–86 (1955)
140. Okabe, N., and Goto, M., Studies on the strains of *Erwinia carotovora* (Jones) Holland, I. Antigenic structure of flagella and their relations to pathogenicity and maltose fermentation, *Bull. Fac. Agr. Shizuoka Univ.*, **6**, 16–32 (1956)
141. Goto, M., and Okabe, N., Studies on the strains of *Erwinia carotovora* (Jones) Holland, III. Mucoid mutation, *Bull. Fac. Agr. Shizuoka Univ.*, **7**, 1–10 (1957)
142. Goto, M., and Okabe, N., Studies on the strains of *Erwinia carotovora* (Jones) Holland, IV, Antigenic variations, *Bull. Fac. Agr. Shizouka Univ.*, **7**, 11–20 (1957)
143. Goto, M., and Okabe, N., Studies on the strains of *Erwinia carotovora* (Jones) Holland, V, Antigenic structures of soma, their relation to biochemical properties, and heat inactivation of agglutinins, *Bull. Fact. Agr. Shizuoka Univ.*, **8**, 1–31 (1958)
144. Novakova, J., A new method of isolation of black-leg pathogens from diseased plants, *Phytopathol. Z.*, **29**, 72–74 (1957)
145. Graham, D. C., Serological diagnosis of potato black-leg and tuber soft rot, *Plant Pathology*, **12**, 142–44 (1963)
146. Starr, M. P., Pectin and galacturonate

metabolism of phytopathogenic bacteria. Intern. Botan. Congr., *Recent Advan. Botany*, 1, 632–635 (1961)

147. Husain, A., and Kelman, A., Tissue is disintegrated. *Plant Pathology, I*, 143–88 (Academic Press, Inc., New York, 1959)
148. Wood, R. K. S., Chemical ability to breach the host barriers, *Plant Pathology*, II, 233–67, (Academic Press, Inc., New York, 1960)
149. Demain, A. L., and Phaff, H. J., Recent advances in the enzymatic hydrolysis of pectic substances, *Wallerstein Lab. Commun.*, 20, 119–40 (1957)
150. Deuel, H., and Stutz, E., Pectic substances and pectic enzymes, *Advan. Enzymol.*, 20, 341–82 (1958)
151. Setterfield, G., and Bayley, S. T., Structure and physiology of cell walls, *Ann. Rev. Plant Physiol.*, 12, 35–62 (1961)
152. Smith, W. K., A survey of the production of pectic enzymes in plant pathogenic and other bacteria. *J. Gen. Microbiol.*, 18, 33–41 (1958); Chromatographic examination of the products of digestion of pectic materials by culture solutions of plant pathogenic and other bacteria, *J. Gen. Microbiol.*, 18, 42–47 (1958)
153. Starr, M. P., and Moran, F., Eliminative split of pectic substances by phytopathogenic soft-rot bacteria. *Science*, 135, 920–21 (1962)
154. Ammann, A., Über die Bildung von Zellulase bei pathogenen Mikroorganismen, *Phytopathol. Z.*, 18, 416–46 (1952)
155. Goto, M., and Okabe. N., Cellulolytic activity of phytopathogenic bacteria, *Nature*, 182, 1516 (1958)
156. Goto, M., and Okabe, N., Studies on the cellulolytic enzymes of phytopathogenic bacteria, Part 1. On the production of Cx-enzyme, Part 2, Relations between the activity of cellulase 'Cx' and pH, reaction temperature or salts, *Ann. Phytopath. Soc., Japan*, 24, 182–88 (1959)
157. Gehring, F., Untersuchungen über den Infektionsverlauf einer durch *Pectobacterium partheni* (Starr) Hellmers var. dianthicola Hellmers verursachten Nelkenbakteriose sowie über enzymatische Eigenschaften dieses Bakteriums im Vergleich mit *Pseudomonas caryophylli* (Burkholder) Starr et Burkholder und einigen typischen Nassfauleerregern, *Phytopathol. Z.*, 43, 383–407 (1962)
158. De Ley, J., Comparative biochemistry and enzymology in bacterial classification, *Microbial. Classification*, 164–95, (12th Symp. Soc. Gen. Microbiol., Cambridge, London, 1962)
159. Le Minor, L., and Ben Hamida, F., Advantages de la recherche de la β-galactosidase sur celle de la fermentation du lactose en milieu complexe dans le diagnostic bacteriologique, en particulier des Enterobacteriaceae. *Ann. Inst. Pasteur*, 102, 267–77 (1962)
160. Paton, A. M., The role of *Pseudomonas* in plant disease, *J. Appl. Bacteriol.*, 23, 526–32 (1960)
161. Sneath, P. H. A., Some thoughts on bacterial classification, *J. Gen. Microbiol.*, 17, 184–200 (1957)
162. Sneath, P. H. A., The application of computers to taxonomy, *J. Gen. Microbiol.*, 17, 201–26 (1957)
163. Sneath, P. H. A., The construction of taxonomic groups. *Microbial Classification*, 289–332 (12th Symp. Soc. Gen. Microbiol., Cambridge, 1962)
164. Colwell, R. R., and Liston, J., Taxonomy of *Xanthomonas* and *Pseudomonas*, *Nature*, 191, 617–19, (1961)

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Effect of inorganic fertilizers on the incidence of potato blackleg disease

by

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EFFECT OF INORGANIC FERTILIZERS ON THE INCIDENCE OF POTATO BLACKLEG DISEASE

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Zusammenfassung, Résumé p. 144

SUMMARY

In assessing disease levels in a number of potato fertilizer trials in south-east Scotland it was found that an increase in the rate of application of complete fertilizer or nitrogen alone resulted in a decrease in the proportion of stems affected by potato blackleg.

1. INTRODUCTION

The attention which has been given to correlation between host plant nutrition and the incidence of attack by pathogenic micro-organisms has been reviewed by GAUMANN (1950) and YARWOOD (1959), especially in the context of host–parasite relations. The evidence which they cite does not support the commonly held opinion that disease susceptibility is favoured by an increase in the supply of nitrogen or a reduction in the supply of potassium (e.g. RUSSELL, 1961 and many elementary textbooks). Once above the nutrient level where deficiency symptoms are manifest, some host–parasite associations show a decrease and others an increase in disease incidence which varies according to the supply of the three major elements, nitrogen, phosphorus and potassium.

Such a relationship has been described for cracking and soft rot of potato tubers caused by the blackleg organism *(Pectobacterium carotovorum* var. *atrosepticum)*, in which an increase in the supply of a complete fertilizer can be accompanied by an increase in the proportion of affected tubers (HARPER et al., 1963). The same organism causes the well known potato blackleg disease affecting the stems, which is responsible for considerable losses in yield in Scotland every year. Because there was little information on the effect of fertilizers on the incidence of blackleg, opportunity was taken to assess the amount of disease in a number of fertilizer yield trials in south-east Scotland during 1963, 1964 and 1965.

2. FIELD OBSERVATIONS

The observations were made on five field experiments laid out in randomized blocks

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or latin square designs, in which the variable was either the rate of application of a concentrated complete fertilizer or of nitrogen alone. The experiments were done on three widely separated farms, the soils varying from a fine sandy loam to a clay loam. Blackleg-infected plants were counted in each plot of each treatment on two occasions, once during mid-season and once near the end, because blackleg develops throughout the whole of the growing period. It was necessary to mark infected plants with canes

Table 1. Effect of concentrated complete fertilizer on blackleg incidence

Year[1]	*Variety and number of tubers planted per treatment*[2]	*Percentage of* N, P_2O_5 *and* K_2O *in fertilizer*[3]	*Fertilizer*[4] *(kg/ha)*	*Percentage blackleg*[5]	
				count 1	*count 2*
1963	Majestic	13 : 13 : 18	0	3.5 N.S.	10.8*
			377	2.1	7.9
	720		753	1.9	5.0
			1,130	0.8	6.2
			1,506	0.4	3.4
1964	Home Guard	13 : 13 : 20	0	3.5 N.S.	11.7**
			377	3.3	7.8
	720		753	1.0	4.9
			1,130	1.1	3.9
			1,506	1.1	3.9
1964	Redskin	10 : 10 : 18	0	3.5*	7.9*
			314	6.8	10.6
	960		628	5.5	9.4
			941	3.0	6.7
			1,255	2.3	7.0
1965	Majestic	17 : 11 : 22	0	1.4 N.S.	9.6**
			377	1.0	10.0
	720		753	1.5	8.2
			1,130	0.4	7.2
			1,506	0.4	5.7

* Significant difference between rates of fertilizer at 5 % level – *signifikanter Unterschied zwischen Düngergaben bei 5 % – différence significative entre les quantités de fertilisants appliqués au seuil de 5 %*
** Significant difference between rates of fertilizer at 1 % level – *signifikanter Unterschied zwischen Düngergaben bei 1 % – différence significative entre les quantités de fertilisants appliqués au seuil de 1 %*
N.S. = not significant – *nicht signifikant – non significatif*
1 kg/ha = 0.892 lb/acre

[1] *Jahr – année*
[2] *Sorte und Anzahl ausgepflanzter Knollen pro Verfahren – variété et nombre de tubercules plantés par traitement*
[3] *Prozent N, P_2O_5 und K_2O im Düngemittel – pourcentage de N, P_2O_5 et K_2O dans la fumure*
[4] *Düngergabe – fumure*
[5] *Prozent Schwarzbeinigkeit (Auszählung 1 und 2) – pourcentage de "jambe noire" (comptage 1 et 2)*

Tabelle 1. *Einfluss von konzentriertem Volldünger auf das Vorkommen von Schwarzbeinigkeit*
Tableau 1. *Effet d'une fumure complète concentrée sur l'incidence de la "jambe noire"*

at the first count to avoid missing those which had died and become buried beneath the foliage of adjacent plants during the time between the first and second counts.

Table 1 summarises the results from the concentrated complete fertilizer trials. These show that, in general, the greater the amount of fertilizer applied the less blackleg occurred. The effect was quite striking, and in two cases less than half as many plants were affected at the highest rate of application as compared with the lowest. Table 2 shows that a similar effect was obtained by increasing nitrogen levels when adequate supplies of potassium and phosphorus were available.

In the 1964 *Home Guard* trial, two identical experiments were laid down side by side, one irrigated with pipes placed in the drills, the other not irrigated. The summer was dry and irrigation frequently applied, but there was no significant difference in the amounts of blackleg between the two treatments. It is also noteworthy that in the 1964 *Redskin* trials, potassium was applied either as sulphate or chloride, but statistical analysis revealed that the form in which the potassium was available did not affect the amount of blackleg.

Table 2. Effect of nitrogen on blackleg incidence, 1963

Variety and number of tubers planted per treatment[1]	N *as* $(NH_4)_2SO_4$ *(kg/ha)*	*Percentage blackleg*	
		count 1	*count 2*
Kerr's Pink	0	17.5**	28.5*
	56	20.5	32.0
330	112	17.5	33.0
	168	14.0	28.0
	224	7.0	20.5
Majestic	0	16.5**	21.5*
	56	14.5	24.0
330	112	11.0	18.5
	168	10.5	15.0
	224	6.5	15.0
Arran Consul	0	7.5**	17.5*
	56	10.0	19.5
330	112	3.0	16.5
	168	2.0	11.0
	224	1.5	12.0

[1] See Table 1 – *siehe Tabelle 1 – voir Tableau 1*
The difference between varieties is significant at 1% level for first count and at 5% level for second count. There is no N × variety interaction, i.e. the three varieties all behave in the same manner – *Der Sortenunterschied ist für die erste Auszählung bei 1% und für die zweite bei 5% signifikant. Es gibt keine Interaktion N × Sorte, d.h. die drei Sorten verhalten sich gleich – La différence entre les variétés est significative au seuil de 1% lors du premier comptage et au seuil de 5% pour le second. Il n'y a aucune interaction "N × variété", c'est-à-dire que les trois variétés se comportent toutes de la même manière.*

TABELLE 2. *Einfluss von Stickstoff auf das Vorkommen von Schwarzbeinigkeit, 1963*
TABLEAU 2. *Effet de l'azote sur l'incidence de la "jambe noire", 1963*

3. DISCUSSION

Stem infection usually takes place when blackleg bacteria in the decomposing mother tuber migrate through the connections between stem and tuber. There must be some mechanism in plants receiving a high plane of nutrition which delays the passage of the bacteria into stems or increases the resistance of the host tissue, or perhaps both. Such plants are taller than those on a low plane of nutrition, have a larger leaf area, a greater bulk of tissue, with a deeper green colour and whereas lower leaves are shed earlier, new leaves are produced over a longer growing period. Thus it seems that so long as plants grow vigorously and photosynthesize actively, they are not so readily attacked. This would account for the fact that the difference in disease incidence between low and high rate plots in the 1963 nitrogen trial (Table 2) was greater at the first count earlier in the season, but later, as the photosynthetic efficiency of high rate plants fell towards the same level as low rate plants, the difference in the amount of disease became less marked. In a number of instances, the amount of blackleg in plots to which no fertilizer had been applied was less than in those which received the lowest amount of fertilizer. This apparently anomalous result is difficult to explain, but it may be that on some soils the nutrient level was so low that plants were suffering a degree of deficiency which reduced blackleg susceptibility, perhaps through lack of readily available food material for the pathogen.

Another aspect of the results is that they help to explain why the amounts of blackleg in plants grown from the same stock of tubers can vary considerably from farm to farm, since the nutrient level in the fields is likely to be different.

One of the most interesting features of the blackleg soft rot complex is that whereas fertilizers decrease blackleg they can increase soft rot in tubers (HARPER et al., 1963), although both are usually caused by the same organism in Scotland. Therefore it could be argued that there should be a certain rate of application of fertilizer where decreases in one disease are counterbalanced by increases in the other in such a way that losses in yield are at a minimum. In practice this cannot be so, because development of tuber soft rot depends not only on fertilizer rate, but also on other factors such as the weather during the growing season, amount of mechanical damage at lifting and the storage conditions.

Observations are continuing on a series of factorial experiments to discover more about how the interactions of nitrogen, potassium and phosphorus influence blackleg incidence.

ZUSAMMENFASSUNG

EINFLUSS ANORGANISCHER DÜNGEMITTEL AUF DAS VORKOMMEN DER SCHWARZBEINIGKEIT AN KARTOFFELN

Der Umfang der Schwarzbeinigkeit an Kartoffeln wurde in fünf Düngerversuchen in Südost-Schottland festgestellt. In diesen Versuchen wurde entweder die Gabe eines konzentrierten Volldüngers oder aber des Stickstoffs allein geändert. Da Schwarzbeinigkeit zu jeder Zeit während der

Wachstumsperiode auftreten kann, wurden zwei Auszählungen vorgenommen, eine in der Mitte und eine gegen das Ende der Vegetationszeit.
Die Ergebnisse der Volldüngerversuche sind in Tabelle 1 und jene des Stickstoffversuches in Tabelle 2 wiedergegeben. Es wird gezeigt, dass im allgemeinen eine Erhöhung der Volldünger- oder Stickstoffgaben eine signifikante Abnahme des Anteils an Schwarzbeinigkeit verursachte, aber die Art und Weise, wie die Düngemittel das Krankheitsvorkommen vermindern, ist nicht klar. Mit Hilfe der Ergebnisse kann eine Erklärung gefunden werden, warum der Umfang an Schwarzbeinigkeit in Beständen mit Knollen gleicher Herkunft von Betrieb zu Betrieb beträchtlich variieren kann. Da der Nährstoffspiegel in den Feldern unterschiedlich zu sein scheint, wirkt sich dies im Ausmass des Auftretens der Krankheit aus.

RÉSUMÉ

L'ACTION DE FERTILISANTS INORGANIQUES SUR L'APPARITION DE LA MALADIE DE LA "JAMBE NOIRE" DE LA POMME DE TERRE

On détermine les manifestations de "jambe noire" dans cinq essais de fumure situés dans le sud-est de l'Ecosse; dans ces essais on fait varier soit la quantité de fumure concentrée complète, soit l'azote seul. Etant donné que la "jambe noire" peut apparaître à tout moment pendant la période de croissance, les comptages de "jambe noire" sont faits deux fois, la première fois au milieu et la seconde vers la fin de saison.
Les Tableaux 1 et 2 donnent respectivement les résultats obtenus avec la fumure complète et avec l'azote. Ceux-ci montrent que, généralement, une augmentation de la quantité de fumure complète appliquée, ou d'azote seul, amène une diminution significative du nombre de tiges atteintes de "jambe noire", mais le mécanisme par lequel les fumures réduisent l'indicence de la maladie n'est pas clair. Ces résultats aident à comprendre comment le nombre de "jambe noire" peut varier considérablement de ferme à ferme dans des récoltes provenant de plants de même origine; en effet le niveau de nutrition dans les champs est probablement différent, ce qui se reflète dans l'importance de la maladie.

REFERENCES

GAUMANN, E. A. (1950): Principles of Plant Infection. English edition. Crosby, Lockwood, London.

HARPER, P. C., BOYD, A. E. W. and GRAHAM, D. C. (1963): Growth cracking and bacterial soft rot in potato tubers. *Pl. Path.* **12**, 139–142.

RUSSELL, E. W. (1961): Soil Conditions and Plant Growth. Ninth edition. Longmans, London.

YARWOOD, C. E. (1959): Predisposition, in Plant Pathology, an Advanced Treatise. Edited by J. G. Horsfall and A. E. Dimond. Acad. Press, New York and London.

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Identity of Gram Negative, Yellow Pigmented, Fermentative Bacteria isolated from Plants and Animals

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Summary. Morphological, cultural and biochemical properties of 35 isolates of rod shaped Gram negative, yellow pigmented anaerogenic, fermentative bacteria were compared. The isolates comprised named cultures of *Bacterium herbicola*, *Erwinia lathyri*, *E. ananas*, *E. milletiae*, *E. uredovora* and *B. typhi flavum*, as well as organisms isolated from deer and man which were considered to be related to *E. milletiae*. The results showed that the organisms were indistinguishable from one another on the basis of the tests employed, and it is concluded that *B. herbicola*, *B. typhi flavum*, *E. lathyri* and *E. ananas* should be classified as *Erwinia herbicola* (Düggeli) Dye. Since there was insufficient information regarding the plant pathogenicity of *E. milletiae* and *E. uredovora*, it is suggested that they should remain separate species at present. The relationship of this group of organisms to certain coliforms and to flavobacteria is briefly discussed.

Rod shaped, Gram negative, yellow pigmented, anaerogenic, fermentative bacteria are common among bacterial isolates made from plants and seeds on ordinary nutrient media. Early bacteriologists were attracted by their colour, and gave them a variety of generic and specific names which ultimately led to confusion regarding the identity of the organisms studied by different workers. The best known name for such organisms among agricultural bacteriologists is *Bacterium herbicola*, a binomial attributed to Löhnis (1911), derived from *B. herbicola aureum* originally introduced by Düggeli (1904). In *Bergey's Manual* (Breed, Murray & Hitchens, 1948), *B. herbicola* is discussed as a possible synonym of *Pseudomonas trifolii*, a name proposed by Hüss (1907), who stated that his isolates had polar flagella and thus were pseudomonads. James (1955) transferred the organism to the genus *Xanthomonas* but retained the specific epithet *trifolii*, whereas Gorlenko (1965) used the binomial *Xanthomonas herbicola*. Plant pathologists, however, are more familiar with *Erwinia lathyri*, first described as the cause of streak disease of sweet peas by Manns & Taubenhaus (1913). Later it was considered the cause of a variety of diseases including tomato streak and chocolate spot of broad bean, but the first two are now known to be virus diseases, whereas chocolate spot is caused by *Botrytis* spp., and *E. lathyri* is generally regarded as a common saprophyte on plants and can also be isolated from soil (Graham, 1958).

Dye (1964), who made a detailed comparison of *X. trifolii* and *E. lathyri* together with several unidentified yellow bacteria from plants, cleared up much of the confusion by showing that *X. trifolii* was not polarly flagellated as was previously believed, but

peritrichously flagellated like *E. lathyri*. Their cultural and biochemical properties were also very similar and Dye suggested that they should be classified as *E. herbicola*.

Two questions remain outstanding. The first is whether the 7 other yellow pigmented species of *Erwinia* reported in the literature as causing plant diseases are similar to *E. lathyri* (Graham, 1958), although the difficulty is that only 3 of these, *E. ananas* (Serrano, 1928), *E. milletiae* (Kawakami & Yoshida, 1920), and *E. uredovora* (Dye, 1963) are still available for comparative study. *Erwinia ananas* was said to be the cause of pineapple fruitlet rot in the Phillipines, whereas *E. milletiae* produced galls on *Milletia floribunda* in Japan. *Erwinia uredovora* was shown to be pathogenic to the uredia of cereal rusts by Pon, Townsend, Wessman, Schmitt & Kingsolver (1954), although the organism was believed to be a xanthomonad by these workers.

The second question concerns the identity of certain anaerogenic yellow bacteria isolated from animals and humans. The first isolations from human clinical material were made by Dresel & Stickl (1928) from cases of typhoid fever in Germany, though later other medical bacteriologists obtained the organisms from numerous sources including blood, urine, water, grass and air. These bacteria were named *B. typhi flavum*, and the particular interest in them lay in the claim that they could be transformed into *B. typhosum* (*Salmonella typhi*) during the course of more or less frequent subculturing on artificial media. The claim stimulated a great deal of controversy in the early 1930's, resulting in studies which demonstrated conclusively that transformation did not occur (Cruickshank, 1935). Wilson & Miles (1955) transferred the organisms to the genus *Chromobacterium* as *C. typhi-flavum* in accordance with their system of classification (although the generic name appeared in inverted commas in the latest edition of their work, emphasizing doubt regarding their taxonomic position (Wilson & Miles, 1964)), but it does not seem to have been realized that *C. typhi-flavum* was apparently similar to *B. herbicola* even though both could be obtained from the same habitats. More recently Muraschi, Friend & Bolles (1965), in characterizing a number of yellow fermentative bacteria isolated from the internal organs of deer and from human throats, did not relate them to *B. typhi flavum*, but stated they were like *E. milletiae*, a culture of which they obtained from The American Type Culture Collection.

This paper describes studies which attempt to clarify interrelationships between the *B. herbicola* group, yellow pigmented species of *Erwinia*, and *B. typhi flavum*.

Materials and Methods

Origin of cultures

Six cultures of *B. herbicola* were received from Dr A. J. Holding, Edinburgh; they were isolated from sources such as silage and oat seed. Another culture from oat seed (D11), described by Duff, Webley & Scott (1963), was supplied by Dr. D. M. Webley, Aberdeen. Of the 6 strains of *E. lathyri*, one each was isolated from Indian rice seed, Scottish oat seed, Tanzanian cowpea seed and English bean seed at East Craigs. One was isolated from rotting bean leaves at East Craigs, and one was received from the National Collection of Plant Pathogenic Bacteria (NCPPB 102). The 4 cultures of *E. ananas* came from Hawaii, and were isolated by Dr. C. H. Spiegelberg from pineapple

rots; the *E. milletiae* culture was isolated from galls on *M. floribunda* in Japan and was received from Dr. M. Goto. All 5 *E. uredovora* cultures were obtained from the National Collection of Plant Pathogenic Bacteria (NCPPB 391, 800, 802, 1044, 1416).

The 5 isolates of *B. typhi flavum* were isolated from human clinical material and were received through the National Collection of Type Cultures (NCTC). Five cultures of yellow organisms isolated from deer and 2 from man were kindly given by Dr. T. Muraschi, Albany, New York.

Incubation temperature

All cultures were incubated at 26° unless otherwise stated.

Cultural characters

Gram staining reaction

Weigert's modification of Gram's method was used.

Motility

This was determined in hanging drop preparations following incubation for 24 h in nutrient broth (peptone (Oxoid L 37), 1·0 g; beef extract (Oxoid), 0·5 g; distilled water, 100 ml; pH 7·2).

Formation of symplasmata

Hanging drop preparations were made from the syneresis water in glucose nutrient agar slopes (nutrient broth plus 0·5% of glucose solidified with 1·5% (w/v) of agar, pH 7·2) after incubation for 24 h. The sausage shaped aggregations of bacteria (symplasmata) were best observed by phase contrast, although they could also be easily seen with direct illumination.

Biconvex bodies in colonies

Bacteria were streaked on nutrient agar plates and incubated for 2–3 days. Biconvex, spindle shaped bodies in the colonies were easily seen with a low power stereoscopic binocular microscope.

Pigmentation

The organisms were grown on nutrient skim milk agar (nutrient broth plus 3·0% (w/v) of skim milk powder (Oxoid) solidified with 1·5% (w/v) of agar, pH 7·2, sterilized at 121° for 5 min). Growth and pigment production were observed after 2–3 days incubation.

Mucoid growth on sucrose agar

Organisms were grown on sucrose agar slopes (5·0% (w/v) of sucrose added to nutrient broth and solidified with 1·5% (w/v) of agar, pH 7·2, for 4 days and examined for production of mucoid growth.

Salt tolerance

Nutrient broth containing 5·0% (w/v) and 10·0% (w/v) of NaCl was inoculated with a 24 h broth culture of the test organisms and incubated 4 days before final readings were taken.

Growth at 37°

One drop of a light suspension of organisms in distilled water was added to nutrient broth filled to a depth of about 5 cm in test tubes and incubated in a water bath for 2 days.

Metabolism of sugars and related compounds

Oxidation-fermentation (O/F) tests

Hugh & Leifson's (1953) medium was dispensed to a depth of about 7 cm in test tubes. Sterile liquid paraffin (B.P. grade) was used as a seal, following stab inoculation of the test bacteria. In another series of tests lactose replaced glucose. Tubes were examined over a period of 4 days.

Utilization of sugars and related compounds

The following compounds were incorporated at 1·0% (w/v) in Dowson's (1957) inorganic basal medium with bromothymol blue indicator; glucose, lactose, maltose, xylose, rhamnose, trehalose, raffinose, adonitol, glycerol, inositol, sorbitol and salicin. Sterilization was by autoclaving at 121° for 1 min. Gas formation was detected with Durham tubes and observations were made over a period of 21 days. Acid production from ethanol was determined using Massey's agar (Massey, 1924) also incubated for 21 days.

Biochemical tests

Reduction of nitrate

This was determined by growing the organism in a medium containing 1·0% (w/v) of peptone (Evans) plus 0·2% of KNO_3 for 2 days and testing for the presence of nitrite with dimethyl α-naphthylamine-sulphanilic acid reagent. Zinc dust was added to cultures giving a negative reaction to determine if nitrate remained and that decomposition had not proceeded beyond the nitrite stage.

Indole production

Cultures were grown in tryptone broth (1·0% of tryptone (Difco) plus 0·1% of tryptophan) for 2 days; the presence of indole was detected with Kovacs' *p*-dimethylaminobenzaldehyde reagent.

H_2S production

A similar medium to that for the indole tests was used; H_2S was detected by lead acetate papers suspended above the medium, and final readings taken after 14 days.

Methyl Red (MR) and Voges-Proskauer (V-P) tests

Organisms were grown in a medium containing glucose, 0·5%; peptone (Evans), 0·5%; K_2HPO_4, 0·5%, for 3 and 5 days. Acetylmethylcarbinol was detected using the caustic potash-creatine method.

Liquefaction of gelatin

A medium containing (w/v) peptone (Evans), 0·5%; yeast extract (Oxoid), 0·1%; gelatin (Oxoid), 15·0%; pH 7·0; sterilized at 121° for 10 min, was dispensed to a depth of 5 cm in test tubes and inoculated by stabbing. Cultures were incubated at 22° for 28 days.

Starch hydrolysis

The medium containing (w/v): peptone (Evans), 1·0%; beef extract (Oxoid) 0·1%; soluble starch (B.D.H.), 0·2%; agar, 1·5%; pH 7·0, was sterilized at 115° for 10 min. Plates were inoculated by streaking and hydrolysis detected by flooding plates with Lugol's iodine solution after 3 days.

Lipolysis

This was observed on plates of tributyrin agar (Oxoid) to which 0·015% of Night Blue indicator was added. Readings were taken after 5 days.

Hydrolysis of cellulose

Skerman's (1959) filter paper strip method was used.

Rotting of potato slices

Slices from clean healthy potato tubers were placed on moist filter paper in petri dishes, inoculated in the centre of the slice with a large loopful of organisms from an agar culture, and examined for rotting after 2 days incubation.

Gluconate test

The medium used was that given by Graham & Dowson (1960) with incubation for 2 days in static culture. A number of cultures were also grown in the same medium, but tested for the presence of 2-ketogluconate with Benedict's reagent after 4 days incubation with constant shaking.

Oxidase test

Steel's (1961) modification of Kovacs' method was employed.

Urease test

Christensen's (1946) agar was used with incubation for 4 days. The same medium without urea was used as a control.

Catalase test

Loopfuls of organisms grown for 24 h on nutrient agar slopes were emulsified with a few drops of 20 vol H_2O_2 in Durham tubes and examined for evolution of oxygen.

ONPG (o-*nitrophenyl*-p-D-*galactopyranoside*) test

Lowe's (1962) method was used.

Utilization of organic acids

Simmon's citrate broth was used for the citrate utilization test. Malonate and acetate utilization was determined using Shaw & Clarke's (1955) medium without phenylalanine, to which 0·5% of sodium acetate or sodium malonate was added. Observations were made over 4 days.

Decarboxylase tests

Møller's (1955) medium was employed, with bromothymol blue replacing bromocresol purple indicator. Final readings were taken after 4 days.

Phenylalanine deaminase test

Organisms were grown on phenylalanine agar (Ewing, Davis & Reavis, 1957) for 2 or 3 days before testing for the presence of phenylpyruvic acid with ferric chloride reagent. The same medium without phenylalanine was also inoculated as a control.

Fourteen of the cultures were also grown for 2 days in phenylalanine broth and the presence of phenylpyruvic acid detected using the dinitrophenylhydrazine reagent (Dickinson & Mocquot, 1961) which gives a red-brown colour with α-keto acids.

Growth in KCN

Test tubes (16×150 mm), filled to a depth of 5 cm with modified Møller KCN medium (Cowan & Steel, 1965) were each inoculated with a loopful of a 24 h nutrient broth culture, tightly closed with rubber stoppers and observed for growth over 3 days. Inoculated tubes of medium without KCN were incubated as controls. To check the results of the visual observations, the growth of one strain of *E. ananas*, one of *E. lathyri*, one of *B. typhi flavum*, and an isolate of *Enterobacter cloacae* (as a control) was followed turbidimetrically with an EEL nephelometer (Evans Electroselenium Ltd., Halstead, Essex, England).

Deoxyribonuclease (DNase) test

Plates of DNase test agar (Difco) were heavily spotted with loopfuls of organisms from nutrient agar and incubated for 2 days. DNase production was detected by flooding plates with N-HCl and observing zones of clearing around growth.

Electron microscopy

Cultures were grown on nutrient agar slopes (Difco) at 20° for 18, 24 and 48 h. The cells were harvested into sterile distilled water in which they were washed three times by slow centrifugation (2500 *g*) and finally resuspended. Droplets of this washed cell suspension were placed on formvar coated grids, air dried and shadowed with gold-palladium (40:60) at an angle of 20°

Preparations were examined in a Siemens Elmiscop I system, using the single condenser system, a 200 μ condenser aperture, a 50 μ objective aperture and an accelerating voltage of 60 kv. Micrographs were recorded at initial magnifications of ×6000–×10,000 diam on Ilford N50 plates.

Results

Most of the results are summarized in Table 1. Those which are not given and those which are of particular interest or require further explanation are discussed briefly below.

Cultural characteristics

Gram stain

All bacteria were rod shaped and unequivocally Gram negative in young (24 h) cultures.

Motility

Motile cells were present in every culture although some cultures contained very few motile organisms.

Symplasmata

Sausage shaped zoogloeal masses of bacteria have been observed in cultures of *B. herbicola* by several workers and were figured by Mack (1936). Medical bacteriologists

TABLE 1

Characteristics of 35 isolates of yellow pigmented bacteria

	Nos. of isolates giving reactions													
	B. herbicola		*B. typhi flavum*		*E. lathyri*		*E. ananas*		*E. uredovora*		*E. milletiae*		Muraschi isolates	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Symplasmata	6	1	4	1	3	3	0	4	3	2	1	0	6	1
Biconvex bodies	6	1	3	2	2	4	0	4	0	5	1	0	5	2
Mucoid growth on sucrose agar	7	0	4	1	5	1	0	4	0	5	1	0	7	0
Growth at 37°	6	1	5	0	5	1	4	0	5	0	1	0	7	0
Acid from lactose	0	7	2	3	3	3	4	0	5	0	0	1	0	7
raffinose	0	7	1	4	1	5	4	0	5	0	0	1	0	7
rhamnose	7	0	5	0	6	0	2	2	5	0	1	0	7	0
inositol	7	0	4	1	6	0	4	0	5	0	1	0	7	0
Acid on ethanol agar	0	7	1	4	2	4	2	2	0	5	0	1	0	7
Reduction of nitrate	6	1	5	0	4	2	0	4	4	1	1	0	7	0
Indole production	0	7	1	4	0	6	4	0	4	1	0	1	0	7
H_2S production	5	2	3	2	5	1	4	0	1	4	0	1	7	0
MR	0	7	1	4	2	4	3	1	1	4	1	0	0	7
VP	7	0	4	1	4	2	2	2	4	1	0	1	7	0
Liquefaction of gelatin	6	1	4	1	6	0	3	1	5	0	1	0	7	0
Lipolysis	0	7	1	4	2	4	4	0	1	4	0	1	0	7
Gluconate test	1	6	2	3	1	5	0	4	0	5	0	1	1	6
Urease test	1	6	1	4	1	5	0	4	0	5	0	1	0	7
Utilization of malonate	7	0	5	0	6	0	0	4	5	0	1	0	7	0
,, ,, acetate	5	2	5	0	6	0	4	0	5	0	1	0	7	0
Phenylalanine deaminase	7	0	4	1	4	2	1	3	2	3	1	0	7	0
DNase production	0	7	0	5	0	6	0	4	4	1	0	1	0	7

All isolates were positive in following tests: Acid from glucose, maltose, xylose, trehalose, sorbitol, glycerol, salicin; growth in 5% NaCl broth; catalase; utilization of citrate; ONPG.

All isolates were negative in following tests: gas from glucose; acid from adonitol; growth in 10% NaCl broth; starch hydrolysis; rotting of potato slices; cellulose hydrolysis; oxidase; lysine and ornithine decarboxylase; arginine dihydrolase; growth in KCN.

also saw them in *B. typhi flavum*, and they were variously described as 'symplasmata', 'Bakterien-verbanden', 'sausage forms' and 'caterpillar formations' (Cruickshank, 1935). They were granular in structure and, when examined under phase contrast, were seen to be aggregates of organisms. Plant pathologists do not seem to have recorded them from *E. lathyri* or *E. milletiae*, but they were present in some isolates of all species except *E. ananas*.

Biconvex bodies

These bodies were first described within colonies of *B. typhi flavum* on agar plates; they are the whetstones (Wetzsteinformen) of Hirsch (1934), who suggested they might be colonies of contaminating bacteria which were still present even after numerous replatings. Cruickshank (1935) remarked that the bodies appeared to be granular structures analagous to symplasmata, although he suggested they were down-growths of the colony into the medium, since this was the most frequent form of colony growing within a solid medium and also because they remained after removal of the rest of the colony with a wire loop.

Although their occurrence was mentioned only by medical bacteriologists, they were found in 6 isolates of *B. herbicola*, 2 of *E. lathyri*, 5 Muraschi cultures and the one *E. milletiae* culture as well as in 3 strains of *B. typhi flavum*, but were absent from *E. ananas* and *E. uredovora*. Cruickshank (1935) stated that he found only one biconvex body in colonies of the cultures he examined, but some of our isolates produced several in each colony whereas in others, most colonies had none at all and the plates had to be searched carefully. They were best seen after 2–3 days incubation.

A strain of *B. herbicola* (D11) which formed biconvex bodies in many colonies was plated on a hard medium made by solidifying nutrient broth with 5·0% (w/v) of agar (Oxoid No. 3). Bodies were not produced on the agar presumably because the bacteria could not penetrate the hard gel; this is another indication that the bodies were down-growths into the medium.

Pigmentation

All cultures were yellow pigmented in the mass on skim milk nutrient agar but there was considerable variation in the degree of pigmentation, some cultures being very pale whereas others were a bright yellow.

Growth at 37°

Some strains grew well at 37° while with others it was rather difficult to determine whether growth had taken place though only 2 were scored as negative. When streaked on nutrient agar plates, several of the slow growing isolates did not produce colonies even after 4 days.

Metabolism of sugars and related compounds

Oxidation-fermentation tests

In Hugh & Leifson's medium all cultures produced an indicator change throughout its depth within 12–24 h in both open and closed tubes, indicating fermentative metabolism of glucose. In the medium where lactose replaced glucose, different isolates gave consistently different reactions. Isolates fermenting lactose included

4 *E. lathyri*, 2 *B. typhi flavum* and all *E. ananas* and *E. uredovora* cultures, while one culture of *E. lathyri* gave an oxidative reaction in the open tube. All other organisms gave negative reactions.

Utilization of sugars and related compounds

All organisms rapidly utilized a range of sugars, sugar alcohols and glycosides. In the sorbitol medium, an acid reaction was produced in 24 h but on further incubation the pH slowly rose again in most cultures until after about 4 days it almost reached neutrality.

Biochemical tests

Liquefaction of gelatin

Cultures liquefied gelatin slowly and none produced noticeable effects until after 7 days. Three were negative after 21 days.

Lipolysis

Most cultures did not hydrolyse tributyrin, but 9 showed weak activity, the blue zone extending into the agar only 2 or 3 mm.

Gluconate test

Graham (1958) reported *E. lathyri* could form small amounts of 2-ketogluconate from gluconate. In Graham & Dowson's (1960) medium, incubated for 2 days in static culture, only 4 of the organisms gave a reaction sufficiently strong to be regarded as definitely positive. Suzuki & Uchida (1965*a*,*b*) stated that *E. milletiae* formed large amounts of 2-ketogluconate from glucose or gluconate in their media after 4 or 7 days of continuous shaking, and Duff *et al.* (1963) found their isolate of *B. herbicola* produced large quantities of the acid from glucose after continuous shaking.

Representative cultures of all 6 species of organisms and one of the Muraschi cultures which were grown for 4 days in Graham & Dowson's medium with continuous shaking before testing with Benedict's reagent, all gave positive tests, although some produced much larger amounts of cuprous oxide precipitate than others, indicating that capacity to accumulate 2-ketogluconate varied from strain to strain.

Oxidase test

None of the cultures gave a positive test in 10 sec but a few produced a violet coloration in *c.* 50 sec.

Urease test

Three cultures gave strongly alkaline reactions in the urease medium after 24 h incubation, though a culture of *Proteus vulgaris* used for comparison gave a positive test in 2 h. No alkaline reaction developed in the control medium without urea.

Decarboxylase tests

None of the organisms produced ornithine or lysine decarboxylase or possessed the arginine dihydrolase system. In this sense they are similar to the type species of the genus *Erwinia*, *E. amylovora* (Martinec & Kocur, 1964) and the soft rot coliform bacteria (Graham, 1964).

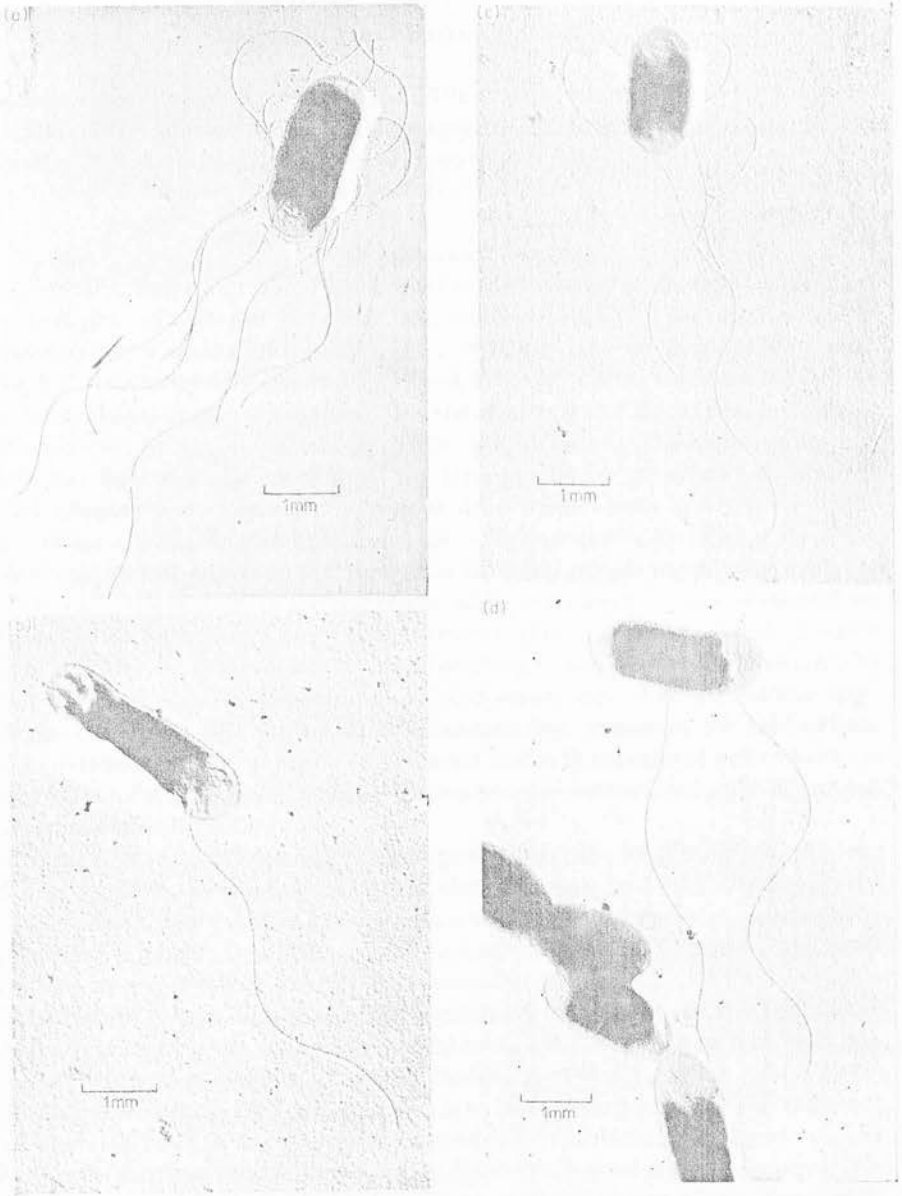

PLATE I. Electron photomicrographs showing the flagella of some yellow fermentative bacteria.

(*a*) *Bacterium herbicola* (Duff, Webley & Scott strain D11), Scottish oat seed. Cell showing several lateral flagella.

(*b*) *Erwinia ananas* (Spiegelberg strain MF 28), isolated from pineapple rot in Hawaii. Cell showing a single, lateral flagellum.

(*c*) *Erwinia lathyri* (Graham strain G157) isolated from English bean seed at East Craigs. Cell showing 3 lateral flagella.

(*d*) *Erwinia lathyri* (Graham strain G146) isolated from Tanzanian cowpea seed at East Craigs. Cell showing 2 lateral flagella.

Flagellation of a strain of *E. uredovora* (NCPPB 800) was studied in detail by Hayward & Hodgkiss (1961). Most of the cells of this organism had 1 or 2 lateral flagella, though occasional cells with more flagella were seen, so that it falls into Group 2.

Flagellation of different isolates is illustrated in Plate 1.

Discussion

Morphologically, culturally and biochemically the isolates of *B. typhi flavum*, *B. herbicola*, *E. lathyri* and the Muraschi isolates showed almost the same range of properties, and it is concluded that all 4 are very closely related. None of the strains of *B. typhi flavum* originally isolated by Dresel & Stickl (1928) nor those studied by Cruickshank (1935) could be obtained, but the characters of the available *B. typhi flavum* cultures corresponded closely with the detailed description given by Cruickshank. Indeed 3 cultures showed the biconvex bodies in colonies on nutrient agar and symplasmata were seen in hanging drop preparations in 4 cultures. The presence of biconvex bodies and the formation of symplasmata by isolates identified as *B. herbicola* and *E. lathyri* as well as by the Muraschi strains was further evidence of the very close relationship of the organisms, although it is not certain whether these structures can be formed by other yellow bacteria which can be isolated from water and plants. However, it is noteworthy that biconvex bodies were not seen in colonies of 8 cultures of *Flavobacterium* spp., 6 of *Cellulomonas* spp., 2 of *Arthrobacter* spp., 3 of *Corynebacterium* spp. nor 16 of *Xanthomonas* spp. examined for comparison. Again, in routine work, the presence of biconvex bodies in colonies of yellow bacteria has proved helpful in making a rapid preliminininary determination of organisms isolated from diseased plants.

The electron microscope studies of flagellation explain the conflicting observations of different workers, some of whom found the organisms to be polarly flagellated (Hüss, 1907; Mack, 1936; James, 1955) whereas others stated they were peritrichously flagellate (Manns, 1915; Dye, 1964). Although certain cells in all isolates had peritrichous flagella, many cells had only a single lateral flagellum, and since it is impossible to discern the exact point of origin of the flagellum in stained preparations examined with an optical microscope, it is understandable that discrepancies in interpretation have arisen (Hodgkiss, 1960; Hayward & Hodgkiss, 1961; Hodgkiss, 1964).

Regarding *E. milletiae*, the results showed it to be indistinguishable from the other organisms on the basis of morphology and on cultural and biochemical properties, so that its only distinctive character is its supposed plant pathogenicity. It was impossible to obtain plants of *Milletia floribunda* in Britain, so the pathogenicity of *E. milletiae* and other isolates could not be tested on this host. Okabe & Goto (1956) stated they had confirmed that it caused galls and Suzuki & Uchida (1965*a*,*b*) illustrated the disease, which resembled a gall such as might be produced by *Agrobacterium tumefaciens*.

Destruction of uredia of cereal rust fungi by *E. uredovora* may involve processes which differ from those concerned in disease production on higher plants and it is not known whether other organisms of the *B. herbicola* group can attack uredia. The capacity of isolates of *E. uredovora* to form extracellular deoxyribonuclease may be related to this property, since the enzyme would presumably destroy DNA within

spores. Since the difficulties regarding pathogenicity have not yet been resolved, it seems best to leave *E. milletiae* and *E. uredovora* as separate species at present.

The nature of the pineapple fruit rot from which Serrano (1928) isolated *E. ananas*, suggests that the condition was not a disease but more akin to generalized tissue decomposition, from which yellow bacteria can often be obtained. As pointed out by Graham (1958), it is possible to produce a variety of symptoms by merely 'inoculating' the fruit with a sterile needle, owing to the presence of organisms in the nectary ducts, blossom cups and even in the glands of the fruitlets. It is to be expected that organisms of the *B. herbicola* group will be present on the tissues, and the process of inoculation will introduce them into the fleshy part of the fruit where they can grow, along with many other bacteria and fungi. In these circumstances, it cannot be claimed that *E. ananas* is truly pathogenic. Morphologically and biochemically, *E. ananas* appears to be distinguishable from most of the isolates of the *B. herbicola* group on the basis of 9 tests, but one isolate of *B. typhi flavum* differed from it in only 3 tests and one isolate of *E. lathyri* in only 4 tests. Thus it seems reasonable to suggest that *E. ananas* should no longer be recognized as a separate species.

As has been pointed out by other workers (Billing & Baker 1963; Dye, 1964), the yellow organisms have properties which resemble those of *E. amylovora*, the type species of the genus *Erwinia*. Although this paper is not primarily concerned with the taxonomy of these bacteria, we agree with Dye (1964) that they may be most suitably included in that genus, and *B. herbicola* and *E. lathyri* should be classified as *E. herbicola* (Düggeli) Dye.* We suggest that *B. typhi flavum* and *E. ananas* should also be classified as *E. herbicola*. It is noteworthy that the soft rot coliform bacteria also have many properties in common with the yellow organisms, but a major difference between them is that only the soft rot coliforms produce pectolytic enzymes. Furthermore no soft rot coliform isolates produce 2-ketogluconate from gluconate (Graham, 1964), whereas *E. amylovora* and *E. herbicola* form 2-ketogluconate in quantity under certain conditions (Duff *et al.*, 1963; Suzuki & Uchida, 1965*a*,*b*). Dickinson (1956) and Billing & Baker (1963) thought that the yellow pigmented organisms might fall into more than one group on the basis of their biochemical properties, but in general our results do not support these suggestions as the isolates gave uniform reactions in many cultural and biochemical tests and showed a scatter of positive and negative reactions in others. However, more isolates would have to be studied before definite conclusions could be drawn.

Gram negative, yellow, fermentative organisms which produce gas from carbohydrates are not common on plants. Thomas & Elson (1957) isolated 57 pigmented coli-aerogenes bacteria over a period of years from soil, water, plants and milk, of which 31 were anaerogenic and may have been similar to the organisms described above. One culture of a yellow bacterium (NCTC 8155) received as *Ent. cloacae* was included in our series of tests. Although it had many of the characters of the anaerogenic isolates it produced gas from glucose, grew in the presence of KCN and gave positive reactions for ornithine decarboxylase and arginine dihydrolase. The value of

* In the *Index Bergeyana* (Buchanan, Holt & Lessel, 1966) the correct name of this organism is given as *E. herbicola* (Geilinger) Dye.

the KCN and amino acid decarboxylase tests as determinative criteria in the examination of Gram negative rods is thus emphasized.

The relationship of *E. herbicola* to other yellow pigmented rods, particularly the flavobacteria, needs clarification. Eight cultures identified as members of the genus *Flavobacterium* received from the National Collection of Industrial Bacteria and the National Collection of Marine Bacteria, were briefly examined for comparison. Six of these were Gram negative organisms which metabolized glucose oxidatively, gave positive oxidase tests, did not utilize citrate, and did not form acid from salicin. However, the 2 remaining organisms slowly metabolized glucose fermentatively (5 days), gave negative oxidase tests, utilized citrate, and produced acid from salicin. One organism was a Gram negative rod whereas the other was a Gram variable rod; both were motile and had many other properties which resembled *E. herbicola*, though neither formed biconvex bodies nor symplasmata, and in general, their production of acid from carbohydrates was slow. It is clear that there are yellow pigmented organisms at present identified as *Flavobacterium* spp. which cannot be readily and confidently distinguished from *E. herbicola*, and further comparative work using a large number of isolates is necessary.

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References

Billing, E. & Baker, L. A. E. (1963). Characteristics of *Erwinia*-like organisms found in plant material. *J. appl. Bact.* **26**, 58.

Breed, R. S., Murray, E. G. D. & Hitchens, A. P. (1948). *Bergey's Manual of Determinative Bacteriology*, 6th ed. Baltimore: Williams & Wilkins Co.

Buchanan, R. E., Holt, J. G. & Lessel, E. F. (1966). *Index Bergeyana*. Edinburgh: E. & S. Livingstone.

Christensen, W. B. (1946). Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bact.* **52**, 461.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Cruickshank, J. C. (1935). A study of the so-called *Bacterium typhi flavum*. *J. Hyg., Camb.* **35**, 354.

Dickinson, A. B. (1956). A study of the Gram negative flora of grass and silage. Ph.D. thesis, University of Edinburgh.

Dickinson, A. B. & Mocquot, G. (1961). Studies on the bacterial flora of the alimentary tract of pigs. 1. Enterobacteriaceae and other Gram negative bacteria. *J. appl. Bact.* **24**, 252.

Dowson, W. J. (1957). *Plant Diseases due to Bacteria*, 2nd ed. London: Cambridge University Press.

Dresel, E. G. & Stickl, O. (1928). Ueber reversible Mutationsformen der Typhusbazillen beim Menschen. *Deut. med. Woch.* **54**, 517.

Duff, R. B., Webley, D. M. & Scott, R. O. (1963). Solubilization of minerals and related materials by 2-ketogluconic acid-producing bacteria. *Soil Sci.* **95**, 105.

Düggeli, M. (1904). Die Bakterienflora gesunder Samen und daraus gezogener Keimpflanzchen. *Zbl. Bakt.* Abt. II **12**, 602; **13**, 56, 198.

Dye, D. W. (1963). The taxonomic position of *Xanthomonas uredovorus* Pon *et al.* 1954. *N.Z. J. Sci.* **6**, 146.

Dye, D. W. (1964). The taxonomic position of *Xanthomonas trifolii* (Hüss 1907) James, 1955. *N.Z. J. Sci.* **7**, 261.

Ewing, W. H., Davis, B. R. & Reavis, R. W. (1957). Phenylalanine and malonate media and their use in enteric bacteriology. *Publ. Hlth Lab.* **15**, 153.

Gorlenko, M. V. (1965). *Bacterial Diseases of Plants* (English translation). Jerusalem: Israel Program for Scientific Translations.

Graham, D. C. (1958). The status of *Erwinia lathyri* and related bacteria. *Comm. Phytopath. News* **4**, 49.

Graham, D. C. & Dowson, W. J. (1960). The coliform bacteria associated with potato black-leg and other soft rots. II. Biochemical characteristics of low- and high-temperatures strains. *Ann. appl. Biol.* **48**, 58.

Graham, D. C. (1964). Taxonomy of the soft rot coliform bacteria. *Ann. Rev. Phytopathol.* **2**, 13.

Hayward, A. C. & Hodgkiss, W. (1961). Taxonomic relationships of *Xanthomonas uredovorus*. *J. gen. Microbiol.* **26**, 133.

Hirsch, W. (1934). Weitere Beitrage zu den Problemen des sogenannten "*Bacterium typhi flavum*". *Zbl. Bakt.* Orig. **131**, 39.

Hodgkiss, W. (1960). The interpretation of flagella stains. *J. appl. Bact.* **23**, 398.

Hodgkiss, W. (1964). The flagella of *Pseudomonas solanacearum*. *J. appl. Bact.* **27**, 278.

Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J. Bact.* **66**, 24.

Hüss, H. (1907). Morphologisch-physiologishe Studien über zwei aroma-bildende Bakterien *Bacillus esterificus* Maasen, und *Pseudomonas trifolii* sp. nov. *Zbl. Bakt.*, Abt. II **19**, 50 and 149.

James, N. (1955). Yellow chromogenic bacteria on wheat. II. Determinative studies. *Canad. J. Microbiol.* **1**, 479.

Kawakami, K. & Yoshida, S. (1920). Bacterial gall on Milletia plant (*Bacillus milletiae* n.sp.). *Bot. Mag. Tokyo* **34**, 110.

Löhnis, F. (1911). *Landwirtschaftlich-bakteriologisches Praktikum*. Berlin: Gebrüder Borntraeger.

Lowe, G. H. (1962). The rapid detection of lactose fermentation in paracolon organisms by the demonstration of *p*-D-galactosidase. *J. med. lab. Tech.* **19**, 21.

Mack, E. (1936). Untersuchungen über *Bacterium herbicola*. *Zbl. Bakt.* Abt. II **95**, 218.

Manns, T. F. & Taubenhaus, J. J. (1913). Streak: a bacterial disease of the sweet pea and clovers. *Gdnrs' Chron.* **53**, 215.

Manns, T. F. (1915). Some new bacterial diseases of legumes and the relationships of the organisms causing the same. *Bull. Delaware agric. Exp. Sta.* no. 108.

Martinec, T. & Kocur, M. (1964). A taxonomic study of *Erwinia amylovora* (Burrill 1882) Winslow *et al.* 1920. *Int. Bull. bact. Nom. Tax.* **14**, 5.

Massey, A. B. (1924). A study of *Bacillus aroideae* Townsend, the cause of a soft rot of tomato, and *B. carotovorus* Jones. *Phytopathology* **14**, 460.

Moller, V. (1955). Simplified tests for some amino acid decarboxylases and the arginine dihydrolase system. *Acta. path. microbiol. scand.* **35**, 158.

Muraschi, T. F., Friend, M. & Bolles, D. (1965). *Erwinia*-like micro-organisms isolated from animal and human hosts. *Appl. Microbiol.* **13**, 128.

Okabe, N. & Goto, M. (1956). Bacterial plant diseases in Japan. VIII. A bacterial gall of the Japanese Wistaria. *Bull. Fac. Agric. Shizuoka Univ.* **6**, 14.

Pon, D. S., Townsend, C. E., Wessman, G. E., Schmitt, C. G. & Kingsolver, C. H. (1954). A *Xanthomonas* parasitic on uredia of cereal rust. *Phytopathology* 44, 707.

Rothberg, N. W. & Swartz, M. N. (1965). Extracellular deoxyribonuclease in members of the family Enterobacteriaceae. *J. Bact.* **90**, 294.

Serrano, F. B. (1928). Bacterial fruitlet brown-rot of pineapple in the Philippines. *Philipp. J. Sci.* **36**, 271.

Shaw, C. & Clarke, P. H. (1955). Biochemical classification of Proteus and Providence cultures. *J. gen. Microbiol.* **13**, 155.

Skerman, V. B. D. (1959). *A Guide to the Identification of the Genera of Bacteria*. Baltimore: Williams & Wilkins Co.

Steel, K. J. (1961). The oxidase reaction as a taxonomic tool. *J. gen. Microbiol.* **25**, 297.

Suzuki, Y. & Uchida, K. (1965*a*). Microbiological studies of phytopathogenic bacteria. I. On 2-ketogluconic acid fermentation by the bacteria belonging to the *Erwinia amylovora* group. *Agr. biol. Chem.* **29**, 456.

Suzuki, Y. & Uchida, K. (1965*b*). Microbiological studies of phytopathogenic bacteria. II. Comparative studies of oxidative fermentation by various species of the genus *Erwinia*. *Agr. biol. Chem.* **29**, 462.

Thomas, S. B. & Elson, K. (1957). Pigmented strains of coil-aerogenes bacteria. *J. appl. Bact.* **20**, 50.

Wilson, G. S. & Miles, A. A. (1955). *Topley and Wilson's Principles of Bacteriology and Immunity*, 4th ed. London: Edward Arnold Ltd.

Wilson, G. S. & Miles, A. A. (1964). *Topley and Wilson's Principles of Bacteriology and Immunity*, 5th ed. London: Edward Arnold Ltd.

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Potato Blackleg and Tuber Soft Rot

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POTATO blackleg is widespread and probably occurs in every country in which potatoes are grown. Under the Scottish seed potato certification scheme it was first considered for all grades in 1932, but it existed in stocks in the 19th century and certainly presents no new problem. Articles written by knowledgeable farmers more than a hundred years ago show that some crops were suffering considerable losses from the condition known as 'curl'. 'Curl' is now generally considered to have been the virus disease leaf roll, but from certain old descriptions of the symptoms it seems likely that many cases were really blackleg. Plainly there was some confusion between the leaf curl caused by leaf roll virus infection and the rolling of the leaves of blackleg-infected plants.

Blackleg is caused by one of a group of bacteria called the soft rot coliforms which affect many kinds of plants throughout the world. These organisms produce enzymes that destroy the pectins binding the cell walls, and both blackleg and tuber soft rot result from this form of tissue disintegration, but soft rot can be caused by other organisms also.

Loss of yield caused by blackleg depends upon the time of the attack and the number of stems affected, but the average reduction is approximately 50 per cent. There is no precise information regarding loss from soft rot in storage, but general experience indicates that it is considerable.

Symptoms

Blackleg. The well-known symptoms can be described as follows. After planting, bacteria associated with the mother tuber cause a soft rot and the organisms pass into the developing stems. Sometimes this occurs so soon after planting that stems never emerge, but usually the first symptoms occur in plants several inches high. Such plants are weak and stunted and the foliage is pale green or yellow. The leaves may be rolled upwards and inwards, or when the weather is dry, they will become wilted and soft. The stem base becomes black or dark brown, which gives the disease the name 'blackleg'.

Infections continue to appear throughout the season, and in large mature plants the basal rot is usually black, wet and soft, sometimes with black streaks running several inches up the stems which usually collapse later. Not all stems growing from one tuber necessarily become infected, but the reason for this is unknown.

Soft Rot in Tubers. Blackleg can destroy young plants before tubers are formed, but when older plants are affected the bacteria pass into the heel ends of the tubers through the stolons. Occasionally the tissue is rapidly attacked and a spreading soft rot disintegrates the tubers in the soil, but in most instances the rot is restricted by barriers of cork cells laid down by the tuber. The dead tissue then dries, leaving a characteristic dark, sunken area at the heel end. However, the pathogen may enter the tuber by routes other than through the stolon. Lenticels, growth cracks or wounds offer access while the

tuber lies in the soil, or later during washing or in storage. Soft rot bacteria also commonly invade tissue affected by such fungus diseases as blight, gangrene, and dry rot, and rapidly complete the destruction started by the fungi.

The tuber provides an excellent food material for bacterial multiplication, and the bacteria cause the starch-containing tuber cells to separate from each other to form a wet mush. Other bacteria—mainly saprophytic—enter and grow in this material converting it into a somewhat darker, foul-smelling pulp.

Pit Rot. If, in storage, conditions become dry soon after bacterial infection has taken place through lenticels, further invasion of tuber tissue may cease and circular, dark, pit-like lesions, $\frac{1}{10}$—$\frac{1}{2}$ in. across, form around the lenticels. Unless two or more coalesce the pits are round, and in this way differ from lesions caused by gangrene or dry rot which are almost always rather variable in shape. This symptom is called pit rot, and whereas pits can be produced by any physical, chemical or biological agent which kills tissue below lenticels, experience shows that restricted penetration by bacteria is a major cause. Sometimes pit rot is conspicuous in tubers washed under pressure in mechanical washing machines, especially where these are fitted with scrubbing rollers.

Factors causing Variation in the Expression of the Diseases

Effect of Tuber Size on Blackleg and Soft Rot. In crops where tubers are affected by a generalised soft rot or blackleg infection at the heel end, most disease occurs in the ware fraction, less in the seed, and least in the chats. There is also some evidence that large tubers are more likely to produce blackleg-infected plants than smaller tubers. Why this occurs is not clear, but it may be partly explained by the fact that larger tubers are more likely to suffer from cracking and mechanical damage, thus allowing bacteria more opportunity to enter tissue.

Planting Diseased and Damaged Tubers. Sometimes good crops can be obtained by planting brock, but there is always a risk involved and, apart from the danger of blanking and poor growth, severe outbreaks of blackleg are always possible. Seed attacked by fungus diseases such as dry rot and gangrene, or with blackheart, or which is slightly frosted, is more likely to produce blackleg-infected plants than sound tubers. This is because the tissues of unhealthy tubers are more easily invaded by the bacteria.

Influence of Fertilisers on Blackleg and Soft Rot. Trials in different countries have shown that the higher the rate of application of fertiliser, the greater is the susceptibility of tubers to soft rot. Under the influence of heavy rain and an excessive application of fertiliser, tubers can attain a high rate of water uptake and consequent fast growth rate without any great increase in dry matter content. The skins, which are then subject to great tension, can split easily, especially if the tuber is moved or hit. In 1962 both growth-cracking and soft rot were widespread and there was found to be a connection between these features and the rates at which fertiliser had been applied. Bacteria from diseased stems and tubers spread through the soil and enter the cracks, setting up soft rot under the very wet conditions. Tubers from plants grown with a

high rate of fertiliser are also more susceptible to mechanical damage, with the result that there are more areas through which bacteria can enter.

On the other hand, with blackleg it has been found that an increase in the rate of application of a complete fertiliser—or of nitrogen alone if adequate supplies of potassium and phosphorus are available—results in fewer affected stems. In some instances the effect can be quite striking, especially during the middle of the growing period, but the difference between rates becomes less marked towards the end of the season. The importance of fertilisers in this respect has only recently been realised and the full significance of their relationship to the epidemiology of blackleg is not yet clear. The mechanism whereby fertilisers prevent the development of blackleg is not known, but the results help to explain why the blackleg content of crops grown from the same stock of tubers can vary considerably from place to place since the nutrient level is likely to be different in each field.

The Disease Cycle

The sporadic occurrence of blackleg often raises the question of the source of infection. Many experiments done at Edinburgh during the last ten years have shown that the blackleg organism dies out in unsterilised soil in a matter of weeks. Furthermore, the organism has not been isolated in bacteriological tests from over 200 soil samples taken in spring from various localities. It seems that, contrary to the opinions of many workers, blackleg bacteria do not form part of the natural flora of the soil and, although enormous numbers are released into the soil from diseased plants and tubers, they do not overwinter there.

The implication of this finding is that soil cannot be an important continuing source of infection, and it is now known that blackleg is largely a tuber-borne disease. Many field and laboratory observations confirm this. In the first place, tubers taken from diseased plants are likely to have become systemically infected with bacteria via the stolons. However, it has been known for some years that tubers taken from apparently healthy plants can produce just as many diseased plants as tubers taken from plants affected by blackleg. From this we can infer that tubers on healthy plants are becoming infected in some other way. It is believed that the tubers and stems of diseased plants in the crop form a major source of infection. Large numbers of organisms pass into the soil near the infectors and spread to tubers on adjacent healthy plants. How far the bacteria move in the soil is not known with any certainty, but the distance will probably vary with soil type, drainage and rainfall. Bacteria have been isolated up to four feet away from the infected plants in a medium loam soil still wet following heavy rain, but it is possible that the bacteria could spread much further under suitable conditions. Thus the soil is the medium through which spread occurs, even if the organisms cannot survive there indefinitely, and blackleg bacteria are therefore said to be soil invaders rather than soil inhabitants. During spells of wet weather the healthy tubers are immersed in a dilute suspension of blackleg bacteria which enter through lenticels and growth cracks. Infection can also be established at lifting time through mechanically damaged areas, the organisms being introduced directly by diggers and other machines.

So far we have seen how the bacteria spread and perpetuate themselves in

the growing crop and at lifting, but spread must also occur after lifting. In badly ventilated stores or pits slightly diseased tubers mixed among the bulk can act as foci of infection, the bacteria spreading to healthy tubers through surface moisture or in fluid running on to adjacent tubers. Other means by which organisms could be transferred from diseased to healthy tubers include grading on dirty riddles or spool graders and storage in contaminated bags or boxes, but there is at present very little evidence to show whether these practices spread infection and, if so, to what extent. However, it is noteworthy that in one experiment done in 1966 three times as much blackleg developed in crops grown from spool-graded or riddled seed as in those hand-picked, the grading having been done in early March after pit storage.

In whatever way tubers are infected, one might expect that soft rot would soon develop as does happen on occasion. But in many cases the incubation period is prolonged, often spanning the whole of the storage season, and tubers show no visible signs of disease. The reasons for the long incubation or 'latent' period are not clearly understood, but it depends at least partly on the humidity and temperature of the store and on the biochemistry of the tuber. In some crops many tubers become infected in this way and through this latent infection the organism persists over the winter. When such tubers are planted and the food materials are translocated to the developing sprout the bacteria become active, rotting the tuber tissue and then entering the stem, thus completing the annual cycle of tuber and stem infection.

Incidence of Blackleg

Annual Variation. As with most plant diseases the incidence of blackleg and soft rot varies between crops and years. No detailed surveys of the extent of infection have been made, but data from the Scottish seed potato certification scheme for the years 1958 to 1966 show that the acreage rejected for blackleg infection was below two per cent each year until 1966. Table 1 gives the percentage of the total acreage entered for inspection which was failed because of blackleg and compares this with the percentage of the total acreage which was rejected for all reasons for the years 1958 to 1966. The tolerance for blackleg was reduced from three per cent to two per cent in 1963, so that the figures after 1962 are not strictly comparable with those of 1962 and before.

TABLE 1

Year	*Percentage acreage rejected for blackleg*	*Percentage acreage rejected for all reasons*
1958	0·28	4·34
1959	0·21	3·59
1960	0·07	4·14
1961	0·18	3·67
1962	0·39	5·46
1963	1·70	5·59
1964	0·54	4·03
1965	1·05	8·80*
1966	6·96	10·59

* 5·42 per cent was rejected for mild mosaic. This presumably resulted from difficulty in seeing mild mosaic during the bright weather at inspection time in 1964, the disease therefore being above normal in 1965.

These data have a limited value because they show the acreage which had a blackleg content above the tolerance level at inspection time, but not the

actual amount of infection in individual crops, which can be high. In addition, blackleg develops continuously throughout the growing period increasing beyond what is to be seen at inspection time. To take one example, in 1963 the blackleg content of a crop of Majestic rose from 3·1 per cent at the end of July to 27·2 per cent in early September. From 1958 to 1966, counts of the number of blackleg plants appearing throughout the season have been made each year in a few crops, mostly of the variety Majestic. The true average percentage of blackleg over these years in these sample crops was 8·6 per cent, the lowest content in an individual crop being 0·2 per cent and the highest 39·1 per cent. Although Majestic is a very susceptible variety, these figures do indicate the importance of blackleg.

Serious outbreaks are usually associated with prolonged periods of wet weather during the growing season which encourages manifestation of the disease, or they reflect a previous wet autumn which allows spread of the bacteria and infection of new tubers. For instance, the high figures recorded in 1963 result from wet weather in the autumn of 1962 and in the summer of 1963. Similarly, outbreaks in the 1930's and 1950's can be correlated with weather conditions.

The Epidemic of 1966. Conditions in 1965 and 1966 favoured the spread of blackleg. After being harvested in a wet autumn the tubers were planted in cold soil where the sprouts developed very slowly and the tuber tissues began to break down. June was exceptionally wet with the result that the bacteria were able to multiply and spread into stems much earlier than usual—indeed, early enough for the growing crop inspections to reveal a very high incidence of the disease. In fact, most of the cases of disease appeared early and by late August or September counts of the total blackleg content were no greater than those recorded in years of normal incidence. Probably the dry weather of July 1966 also helped towards the early appearance of symptoms; affected plants were less able than healthy ones to withstand the mild drought.

Control Measures

No British potato variety is resistant to soft rot or blackleg, and so far no attempt has been made to breed for resistance. Satisfactory control cannot be achieved by chemical disinfection of tubers with solutions of such substances as sodium hypochlorite or mercury compounds; nor can blackleg in the growing crop be reduced by spraying with antibacterial preparations.

Control measures must therefore be based on agronomic practice and should aim at reducing the spread of the organism in the growing crop by roguing out infected plants, attempting to prevent the organism from entering tubers at lifting, grading and during storage, planting only healthy, sound tubers, and providing good soil conditions for plant growth.

Roguing. Because bacteria are released from infected stems and rotten tubers and spread through the soil, roguing out affected plants will not completely eradicate the disease. However, the degree of control will depend on the time and frequency of roguing. If this is begun as soon as symptoms appear and is continued regularly throughout the season, it will reduce disease incidence by cutting down the number of organisms in the soil and by eliminating tubers attached to infected plants. Roguing before inspection is limited under the requirements of the certification scheme, but recently the

roguing tolerance was increased to encourage growers to rogue more thoroughly. Sometimes roguing is not followed by much improvement in the following season, but if it is persevered with over four or five years the amount of disease should be reduced. The virus-tested stocks of the Department of Agriculture and Fisheries have been rigorously rogued for blackleg since 1964 and very few affected plants were found in stocks in 1966 and then only in certain clones.

Repeated roguing of large acreages is hardly practicable, but one way of avoiding this difficulty is to have a nursery plot set aside each year to provide seed for home planting, and a special attempt should be made to keep this plot free from blackleg. The grower of virus-tested seed is admirably placed to do this since all his stocks are raised from small nursery units, but any grower can use the nursery plot method to improve the general quality of his crops as well as to reduce blackleg infection. Roguing means that all tubers of affected plants as well as the haulm should be dug up and removed from the field.

It may be asked what part the certification scheme plays in the control of blackleg, especially since the disease is unlikely to be fully manifest in the crop at inspection time. Clearly it is limited, but crops with the greatest amount of blackleg in them at inspection are generally also those which have the greatest total blackleg content. By eliminating the worst crops the scheme does make a practical contribution towards restricting the impact of the disease. Again, blackleg has to be considered in the scheme because the presence of large amounts could interfere with inspection for other diseases.

Preventing Tuber Infection. A determined effort should be made to reduce mechanical damage at lifting time and at grading so that bacteria get the minimum opportunity of entering tissue beneath the skin. The correct use of an efficient haulm-destroying chemical, followed by an adequate period between burning down and lifting to allow skins to mature, is important in this respect.

Riddles which are used for grading should be rubber-coated, and all riddles and spool graders should be thoroughly washed periodically. Cleaning is especially important if there are rotted tubers in the stock being graded, because the machines become smeared with slime containing millions of organisms which might infect healthy tubers.

Penetration of tubers by bacteria through lenticels or damaged areas can be prevented to some extent by dry, well-ventilated storage conditions. Rapid drying out of tuber surfaces soon after lifting prevents many surface-borne organisms from establishing infection within tubers, and experience shows that tubers boxed at lifting produce fewer blackleg-infected plants than tubers stored in pits. Bad storage conditions increase the risk of soft rot, when the organisms are liable to spread to adjacent tubers in storage and will later contaminate equipment such as graders, bags and boxes.

Planting Sound Seed. Cut seed, frosted or badly damaged tubers, and seed attacked by fungus diseases should never be used for planting. It should also be remembered that there is evidence that small tubers are less likely to produce blackleg-infected plants than larger tubers.

Providing Good Soil Conditions. This subject is outside the scope of this article, but plainly fertilisers should be applied at the rates recommended for

the particular area of Scotland where the potatoes are being grown so that plants are not predisposed to disease development. Good drainage is essential because it is well known that blackleg is much more common in parts of fields where drainage is poor. The wet soil accelerates the breakdown of the mother tuber and enables the bacteria to spread freely.

Blackleg-free Stocks

At the present time the grower inevitably has to deal with stocks which are carrying bacterial infection to a greater or lesser extent and for that reason any control measures adopted are only palliative. The ultimate solution to the blackleg problem would probably be to produce stocks that are blackleg-free. How can this be achieved? Recent research on many potato tuber diseases has emphasised the importance of the mother tuber as the major source of over-wintering disease organisms and there is much evidence to show that blackleg is one of these. It follows that if stem cuttings are used to propagate a new generation of tubers these should be free from infection provided the disease does not spread upwards into the stems from the underground parts; the organisms will remain behind in the mother tuber and the surrounding soil. However, blackleg bacteria do spread from the mother tuber into the stem, so that cuttings have to be tested individually for their presence and found free before being used for propagation. Testing involves culturing for bacteria from small portions of stem taken from the base of the cutting, and this requires the facilities of a bacteriological laboratory with skilled staff. Private growers would thus have great difficulty in producing blackleg-tested plants. Nevertheless, the method is feasible and indeed is already in use on some very large nurseries producing stocks of carnation cuttings free from certain bacterial and fungal diseases.

The Department of Agriculture and Fisheries are beginning experiments in raising quantities of tubers grown from tested cuttings, using varieties from virus-tested stocks. To avoid contamination as far as possible, cuttings are being propagated in glasshouses under strict hygienic conditions and the resulting tubers are grown on a farm where there are no other potato stocks.

No one knows exactly what practical difficulties may arise, or how long healthy stocks will remain so on farms where infected stocks are also grown. In fact, the position is in some ways similar to the situation which existed when the testing scheme to eliminate potato virus X (mild mosaic) was first begun, but it is believed that the attempt to produce blackleg-free stocks on a farm scale is worthwhile. Only further experience will show whether the attempt is likely to prove successful, but it seems that in the long term it may be possible to eliminate blackleg from many stocks. Such an achievement would contribute significantly towards the continuing improvement of Scottish seed potatoes.

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Control of Potato Gangrene and Skin Spot Diseases by Fumigation of Tubers with Sec-butylamine

FUNGAL diseases of potato tubers can cause considerable losses in the United Kingdom every year. At present in Scotland the most serious is gangrene, a rot of tuber flesh caused by *Phoma exigua* var. *foveata*. Skin spot, caused by *Oospora pustulans*, is also important, but in this case the organism does not rot tuber flesh: small pustules appear on the surface and, more importantly, eyes may be killed and sprouting affected.

These diseases have proved difficult to control chemically, and until very recently this has only been achieved by disinfecting tubers in solutions of organo-mercury compounds[1]. Mercury treatment has been adopted commercially, but it suffers from several practical difficulties, and although used for many years has made only limited impact on the seed trade. Experimental use of the systemic benzimidazole fungicides is giving promising results, but these still have to be applied to tubers as dusts or in dipping solutions[2].

Gaseous treatment of tubers has not often been investigated, although formaldehyde gas has been tried unsuccessfully. Nevertheless, fumigation has attractive possibilities especially because gases could be introduced into bulks of stored tubers fairly easily. We tested several gases against pure cultures of gangrene and skin spot fungi but substances that seemed promising fungicides proved phytotoxic to tubers. Although *sec*-butylamine (2-aminobutane) had only a limited effect on growth of pure cultures, we decided to see whether it would control natural tuber infections. This substance and its salts have been shown to be actively fungistatic by Eckert and Kolbezen[3], who used them chiefly against fungal rots of citrus fruits. *Sec*-butylamine is a colourless liquid with an ammoniacal odour, easily vaporized (boiling point 63° C), miscible with water and most organic solvents. It is a moderately toxic substance, the toxicity being due primarily to its strong alkalinity, but effects are minimized by both dilution and neutralization.

In the first tests 38 kg of freshly lifted naturally infected tubers of several cultivars were fumigated with different doses of gaseous *sec*-butylamine in a 400 l. steel fumigation

Table 1. EFFECT OF TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF GANGRENE

Cultivar	Dosage (mg/kg) *sec*-butylamine	No. of tubers examined	Per cent gangrene
Majestic, stock 1	85	220	6·1
	140	227	4·8
	Nil	195	33·3
Majestic, stock 2	140	172	4·7
	Nil	171	18·1
King Edward	140	278	2·5
	350	288	4·2
	Nil	259	25·9

Table 2. EFFECT OF TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF SKIN SPOT (CULTIVAR KING EDWARD)

		Percentage of tubers affected in each category							
		Eye infection score				Skin cover score*			
	Dose (mg/kg)	Free	Some	All	None	Trace	Slight	Moderate	Severe
Stock 1	70	84	16	0	68	30	2	0	0
	120	76	24	0	66	26	8	0	0
	Nil	24	48	28	10	28	58	4	0
Stock 2	140	98	2	0	92	6	2	0	0
	350	100	0	0	98	2	0	0	0
	Nil	66	24	10	30	36	20	14	0

* Trace, 1–10 pustules per tuber; slight, up to 1/10 of tuber surface covered; moderate, between 1/10 and 1/4 surface covered; severe, more than 1/4 surface covered.

chamber fitted with an external circulatory system to assist application and distribution of the gas. The potatoes were exposed to the gas for about 2 h during which most of it was absorbed.

The treated and untreated tubers were placed in wooden sprouting trays and stored over winter. All the tubers were examined periodically until the end of March for the appearance of gangrene lesions, and skin spot was assessed at the end of the storage period by Boyd's method[4] using fifty tubers. Results of some of the experiments are shown in Tables 1 and 2. Because the treatments showed such promising control of both diseases with no evidence of tuber phytotoxicity we built a large experimental fumigation chamber to hold 5 tons of tubers, fitted with a forced draught gas recirculation system incorporating an apparatus capable of vaporizing the required amount of *sec*-butylamine in approximately 40 min. Tubers were fumigated within 48 h of lifting with a dose of 200 mg/kg. Analyses of gas samples drawn through tubes ending at different positions in the bulk showed that even distribution of the gas throughout the air spaces between tubers was only obtained slowly. During the application considerable condensation and adsorption took place on the lower layers of tubers, and this was apparently followed by processes such as evaporation, desorption and further adsorption throughout the bulk when recirculation of the

Table 3. EFFECT OF BULK TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF GANGRENE

Cultivar	Treatment	No. of tubers examined	Per cent gangrene
Majestic	200 mg/kg	1,328	0·2
	Nil	1,376	4·1
Redskin	200 mg/kg	567	0·7
	Nil	552	88·6

Table 4. EFFECT OF BULK TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF SKIN SPOT

Percentage of tubers affected in each category

Cultivar	Treatment	Eye infection score			Skin cover score				
		Free	Some	All	None	Trace	Slight	Moderate	Severe
King Edward	200 mg/kg	100	0	0	100	0	0	0	0
	Nil	13	56	31	0	20	70	9	1
Majestic	200 mg/kg	100	0	0	98	2	0	0	0
	Nil	14	76	10	2	32	46	18	2

mixture of air and gas was continued after all the fumigant was applied. Residue analyses on samples taken from various positions in the bulk indicated that good distribution of the fumigant on tubers had been achieved after recirculation for a further 2 h. After fumigation ½ ton bulks made up of tubers taken from different positions in the chamber were stored under straw in a shed until February, when they were sorted and assessed for rot diseases. The healthy material was then boxed. Further disease assessments were made in early April, and the visual diagnoses checked by isolation of fungi from representative tuber samples. For controls ½ ton untreated lots from the same consignment were stored similarly. Results of four experiments are shown in Tables 3 and 4. As well as a visual examination for skin spot, eye plug samples from the King Edward stock were incubated in moist chambers and examined microscopically for the development of *O. pustulans*[5], which was not detected. The tuber diseases dry rot (caused by *Fusarium caeruleum*) and blight (caused by *Phytophthora infestans*) were not controlled, but the effect of fumigation with *sec*-butylamine on other diseases has not yet been investigated.

Sec-butylamine in a dose of 200 mg/kg had no phytotoxic effect on undamaged tubers although badly skinned areas, especially on immature tubers, could be discoloured. Extensive skin damage and complete death of eyes were caused by a dose between 1,000 and 5,000 mg/kg. Preliminary results of field trials showed that treatment of tubers with *sec*-butylamine at 200 mg/kg had no adverse effect on plant growth or yield. Experiments are continuing on various aspects of the treatment including determination of residues in treated tubers and in crops grown from treated tubers. Studies are also being made on the physical processes operating in fumigating bulks

of tubers with *sec*-butylamine. United Kingdom and Irish patents have been applied for.

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[1] Boyd, A. E. W., and Penna, R. J., *Proc. Fourth Brit. Insecticide and Fungicide Conf.*, 1, 294 (1967).
[2] Hide, G. A., Hirst, J. M., and Griffith, R. L., *Proc. Fifth Brit. Insecticide and Fungicide Conf.*, 2, 310 (1969).
[3] Eckert, J. W., and Kolbezen, M. J., *Phytopathology*, 54, 978 (1964).
[4] Boyd, A. E. W., *Ann. Appl. Biol.*, 45, 284 (1957).
[5] Hide, G. A., Hirst, J. M., and Salt, G. A., *Ann. Appl. Biol.*, 62, 309 (1968).

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PROSPECTS FOR CONTROL OF POTATO BLACKLEG DISEASE BY THE USE OF STEM CUTTINGS

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Summary Potato blackleg disease, caused by Erwinia carotovora var. atroseptica is widespread and important in Scotland. Experience has shown it cannot be satisfactorily controlled by chemical treatments, agronomic methods or ordinary certification procedures.

Research has demonstrated that the blackleg organism is essentially tuber-borne and on this is based a method for producing seed free of blackleg and certain other diseases by propagating from tested stem cuttings. These uninfected stocks, called VTSC (Virus Tested Stem Cutting) seed, are now the highest grade in the Scottish Seed Potato Certification Scheme.

In 1970 the first VTSC stocks were released into commerce and in 1971, 210 acres of VTSC crops were certified, no blackleg having been found in them. As expected, reinfections have occurred fairly, widely, but the levels of infection were very low. Nevertheless, as the old infected stocks are continually replaced by material derived from stem cuttings, and farm hygiene improved, the general level of blackleg infection should be greatly reduced.

INTRODUCTION

Unlike most potato diseases, potato blackleg, caused by the soft rot coliform bacterium Erwinia carotovora var. atroseptica cannot be satisfactorily controlled by chemical treatments, ordinary certification procedures or agronomic means. Graham and Harper (1967) briefly summarised the epidemiology of the disease and control measures, but they emphasised that these measures were only palliative because tubers in all stocks were carrying infection to a greater or lesser extent and that the ultimate solution would probably be the production of blackleg-free stocks.

The key to freedom from blackleg is the fact, still unappreciated by many, that the causal organism is not part of the natural bacterial flora of the soil, but is tuber-borne. The following evidence that the blackleg organism is not a soil inhabitant in Scotland is based on research begun 20 years ago at East Craigs.

1. Blackleg bacteria could not be isolated from soils in spring just before potatoes were planted.

2. Populations of blackleg bacteria in both artificially inoculated and naturally contaminated soils declined to undetectable levels in a matter of months.

3. Soil bacteria, though not normally inhabitants of water can nevertheless be water-borne, and it was considered that if the blackleg organism were soil-living, it would occur from time to time in the water of ponds and streams. However, the organism could not be isolated from ponds or streams in arable areas.

4. Soft rot coliform infections of hosts other than potato are very rare in Scotland. If the organisms were soil inhabitants, infections should be comparatively common.

On the other hand there is good evidence that blackleg is tuber-borne; for example the organism can easily be isolated from tubers taken from the great majority of potato stocks. The infection cycle begins with the breakdown of the mother tuber, partly by blackleg bacteria. Thus enormous numbers of organisms invade the soil, and come into contact with the daughter tubers, infecting them through lenticels and growth cracks. They can also spread far enough to infect tubers on adjacent plants. Some daughter tubers can be infected via the stolons, but this mode of infection plays only a very minor role in the perpetuation of the disease. By whichever way they are infected, some tubers rot, but the great majority show no symptoms, and the incubation period is prolonged, often spanning the whole of the storage period. When tubers are planted and begin to grow, the bacteria become active, rotting the tuber, which again releases bacteria into the soil, thus completing the annual cycle of tuber infection.

THE PRINCIPLE OF DISEASE CONTROL THROUGH USE OF STEM CUTTINGS

Recent research on a number of potato tuber diseases has emphasised the importance of the mother tuber as the major source of over-wintering disease organisms, and that the infection cycles initially involve underground parts of the potato plant. It follows that if stem cuttings, detached before organisms spread to them, are used to propagate a new generation of tubers, these tubers should be free from infection. However, since blackleg bacteria might spread quickly from the mother tuber into the stem, cuttings have to be tested bacteriologically to ensure they are uninfected.

Use of tested cuttings for production of healthy plants is not new. For instance, it has been developed on some very large nurseries growing carnation cuttings free from certain fungal and bacterial diseases. The technique was first applied to potatoes at Rothamsted Experimental Station, where it was shown that plants could be freed from the fungus disease skin spot (caused by Oospora pustulans) by this method (Hide, Hirst and Griffith, 1969). There is now evidence that nuclear stocks of tubers free from gangrene (Phoma exigua var. foveata) and silver scurf (Helminthosporium atrovirens) can be produced in the same way. The technique for control of blackleg by using stem cuttings was developed at East Craigs.

STEM CUTTINGS IN PRACTICE

In 1967, the Department of Agriculture and Fisheries for Scotland (DAFS), aware that the problem of tuber diseases was acute, decided that available evidence justified the initiation of a project to produce seed free from blackleg and other diseases using stem cuttings from their existing virus-tested stocks. This pilot scheme proved successful and in subsequent years output was stepped up, the eventual aim being to replace existing commercial stocks with material derived from stem cuttings. No one knew exactly what practical difficulties might arise, or how long stocks might remain healthy, but all evidence indicated that the technique had great potential value in countering tuber diseases. To avoid reinfection of the stocks, the DAFS raises and multiplies the material on an upland farm where no commercial crops are grown, and under strict hygienic conditions. Stocks propagated clonally for up to five years from stem cuttings now constitute the highest grade in the Scottish Seed Certification Scheme and are designated "VTSC" (Virus-Tested Stem Cutting) seed. VTSC clones from the DAFS farm were first released to commercial

VTSC producers in 1970. Crops entered for this grade are very rigorously checked for both purity and health and, under the terms of the Scheme, there is no tolerance for blackleg in the growing crop, or for soft rot caused by the blackleg organism in stored tubers.

Production of VTSC Seed on the DAFS farm. Tubers derived from stem cutting material are potted in a sterile peat compost in 6 in. pots under glass in early spring and when stems are 12 in. high the growing point is pinched to cause the buds in the leaf axils to grow into side shoots. When the shoots are about 3 in. long they are removed aseptically. A piece of stem about ½ in. long is cut from the base of lowest cutting on the main stem, and sent to the laboratory for bacteriological testing. It is assumed that if the lowest cutting is free from blackleg bacteria then those higher up the stem will also be free, since the bacteria spread upwards in the stem. The cuttings from each parent plant are wrapped separately in moist absorbent paper and kept separately in small plastic cups until the results of the tests are known, normally within two days.

Cuttings proved to be blackleg-free are rooted in mist propagators, transplanted in 3 in. pots, hardened off in frames and hand planted in the field in June and July. Tubers of commercial size are produced from cuttings of most varieties by the end of the season. Spent haulm is destroyed chemically and tubers lifted carefully for storage. These tubers are used for clonal multiplication in subsequent years.

The Blackleg Test. The pieces of stem for testing are crushed with sterile pliers and the sap spread over plates of MacConkey pectate double layer medium (Stewart, 1962) which are incubated at 25° for 48 hours. On this medium, blackleg organisms form discrete colonies in depressions in the pectate layer, and, if necessary their identity can be confirmed by slide agglutination tests using antiserum prepared against the organism or by biochemical tests.

Over the five-year period 1967-71, 9,300 cuttings have been tested and none have been found infected, probably because the bacteria had not been able to multiply in the mother tuber and spread into the stem by the time the cuttings were taken. However, cuttings from ordinary commercial stocks examined in July in connection with research work have quite often been found to be infected, though the plants from which they were taken showed no symptoms, and for this reason no cuttings are used for routine propagation after mid-June and never from field plots. Some cuttings are contaminated with large numbers of saprophytic bacteria including pectolytic pseudomonads, and these cuttings are discarded as a matter of routine. The average discard rate over the five-year period was 3.7%.

Blackleg and Stem Cutting Material, 1967-70. Over the years 1967-70 approximately 600,000 plants were grown on the DAFS farm and only one plant, of cv Redskin, was found infected. This was detected after lifting as a heel end tuber infection in material grown two years from the cutting stage, but the source of infection could not be discovered. Another 84 samples of suspect tuber material were examined bacteriologically but proved to be uninfected.

In 1970 sufficient VTSC seed was released to commercial growers to plant 44 acres. There were only three instances where this commercial material was found infected and these were investigated to try to discover how infection had been introduced. In the first case one infected plant was found in a crop which appeared to have been attacked by rooks. In the second case seven plants were found of which five had typical blackleg infection arising from the mother tubers; the other two showed stem infections at soil level but the mother tubers were sound and blackleg bacteria could not be isolated from their interiors. The source of infection was not found; it was noteworthy that the infected plants were in the same strip of plots and in an area which had been kept very wet by overspill from a stream above the field where the plots were growing. The third case was particularly

interesting as it demonstrated that blackleg could be introduced easily with contractors' contaminated spraying equipment. The symptoms were soft rot on stems at places where they had been damaged. Nine plants were infected, all adjacent to the wheel tracks, and investigation showed the machinery had been used previously in crops affected with blackleg to the extent of 3-5%. It was claimed that the equipment had been pressure-hosed before use in the crop, but this had apparently not been sufficient to remove infection.

The tubers from all VTSC crops were inspected during storage and only eight stocks contained soft rotted tubers or tubers with heel end necrosis. Two tubers from one of these stocks and 15 from another were found to be infected but the sources of infection could not be discovered.

Blackleg on the DAFS farm in 1971. In 1971 blackleg was more extensive in commercial seed crops in Scotland than in any year since the epidemic of 1966, presumably because weather conditions were favourable for manifestation of infection.

For the first time the disease was discovered spasmodically in tested cuttings planted in the field. Altogether 21 infected stems were found in early September distributed throughout an area which contained 4,000 plants. From the symptoms it was clear that two plants with stem lesions had been infected several weeks previously, and some tubers on these plants showed extensive internal rots caused by the blackleg organism. Both plants were next to wheel tracks suggesting that infection might have been introduced on the wheels. The remaining infections were either on stems at or above soil level or on exposed leaf scars and apparently had been established more recently because lesions had not spread extensively in the stems. The foliage had suffered severe wind damage and due to the exposed situation and the prevailing wet weather, the haulms were senescent and rotting. The rotting material attracted large numbers of insects, mainly fruit flies (Drosophila sp), which were very active in the crop. A number of flies were collected and tested for contamination with blackleg organisms by plating material from crushed bodies; two collections were found to be contaminated and it seems possible that the insects had been responsible for spreading infection.

A further 15 plants were found infected amongst material grown from tubers derived from stem cuttings in previous years. An examination to try to determine the source of infection is in progress, but not all infections originated from the mother tubers. All blackleg infected stocks have been destroyed.

Blackleg in commercial VTSC crops in 1971. Data on blackleg found at inspection in stocks grown by commercial VTSC growers is as follows:

Total number of stocks	Number of stocks rejected for any blackleg	Total acreage entered for VTSC grade	Acreage rejected for any blackleg	Acreage rejected for other reasons
241	52	270	64½	5½

Twenty-four stocks had levels of infection between 0.001% and 0.01% of the plants, 17 stocks had levels between 0.01% and 0.1% and 11 stocks had levels between 0.1% and 0.5%. Of the 49 growers, 27 had all their stocks free from blackleg, despite the fact that the majority were still growing other crops not derived from stem cuttings.

Investigations are still continuing into sources of infection and means of recontamination, but it is already clear that contaminated machinery is important. There is also circumstantial evidence that some blackleg plants developed from tubers attacked by rooks earlier in the season. Rook damage is quite common in certain areas in mid-summer when food is scarce; and it may be that rooks transmit infection by feeding first in ordinary commercial crops and then in VTSC crops.

Problems of preventing reinfection. The data on the occurrence of blackleg in VTSC stocks illustrates that reinfection takes place fairly commonly at present, but the levels of infection are generally very low - much lower than in crops not of stem cutting origin. Nevertheless once the organism has been introduced infection is likely to build up rapidly; hence there is no tolerance for blackleg in VTSC material.

No one should be surprised at the extent of reinfection, considering the amount of infection in all other commercial stocks and the many ways in which organisms could be transferred to blackleg-free stocks. Some of these ways seem obvious; for example the use of contaminated planters, sprayers, tractors, lifting machines and storage equipment, but there is very little experimental evidence of the extent to which these practices spread disease. Experiments have been done at East Craigs on reinfection of VTSC tubers by using a contaminated riddle and spool grader. The graders were contaminated by passing over them tubers with soft rot caused by the blackleg organism. Two treatments were used: VTSC tubers graded immediately after contamination, and after some contamination had been removed by passing 5 cwt healthy tubers over the grader before the VTSC tubers. Grading was done in early March and tubers were stored in trays until planting in April. The results are shown in Table 1.

Table 1

Reinfection of blackleg-free stocks of cv. Arran Pilot by grading

Type of grader	% blackleg appearing in subsequent crop following	
	1. Dressing over contaminated grader	2. As 1. after 5 cwt. healthy tubers had passed over the grader
Spool	16%	2%
Riddle	19%	11%

These results demonstrate the high infectivity of the organism and the necessity for very good hygiene on the farm. Disinfection of all equipment including clothing is essential, but a major practical problem has proved to be the cleaning and disinfection of machinery, which, of course, is not designed with disinfection in mind. Tests have shown that infective material remains lodged in such places as junctions between boards and nut and bolt heads, even after pressure washing. To seal up these sites as well as to provide an easily cleaned surface growers are recommended to paint machinery with a chemically resistant thick paint.

Tests have also shown that many commercial disinfectants such as quaternary ammonium compounds, hypochlorite and some chlorinated phenols are ineffective because they are too readily inactivated by contact with soil and plant debris. The most effective disinfectant found so far is 5% formaldehyde solution containing a wetting agent, but in view of the unpleasant properties of formaldehyde, experiments are continuing at East Craigs to find an odourless, non-toxic, non-corrosive alternative.

Another obvious source of contamination is groundkeepers, but there is no experimental evidence to show how important they are in this respect. However an eight-year rotation is laid down for VTSC crops, and this should greatly minimise the danger from groundkeepers. There is no satisfactory way of destroying them quickly and the development of a herbicide which translocates from foliage to tubers *in situ* would be a valuable aid in the control of a number of tuber-borne diseases.

Experience with blackleg-free material is bringing to light other possible means of re-infection which were completely obscured by tuber-borne infection. Transmission by rooks is an example, and experiments to test this hypothesis will begin in 1972. If rooks do spread blackleg it is difficult to see how it can be stopped; but it may go some way to explain how crops can become infected despite every reasonable hygienic precaution having been taken.

PROSPECTS FOR THE FUTURE

It would be naive to imagine that merely to take cuttings and propagate from these will, even in the long term, suffice to eliminate blackleg. Stem cutting production is only a first step, but at this stage of development, where islands of disease-free material are being grown in a sea of infection, re-infections are bound to occur. Nevertheless, as old infected stocks are continuously replaced with material derived from stem cuttings, and hygiene improved, the level of blackleg infection should be greatly reduced. Eventually, the disease should no longer cause so much loss and trouble even if it is not entirely eliminated. Dilution of infection should be accelerated by new requirements under the Scottish Certification Scheme, which limit the life of VTSC stocks and the next highest grade "FS" (Foundation Seed) to five and four years respectively. Thus there will be a continuous downward flow of high grade material through the grades. Coupled with these conditions the Department are carrying out a vigorous educational programme for growers by direct advice, through publications and other forms of publicity.

Unlike some of the fungal diseases, an additional problem in blackleg control stems from the fact that hygiene is the only means preventing re-infection. Skin spot and gangrene, for instance, can be controlled by the systemic fungicides and by fumigation with sec-butylamine, so that combining by chemical treatment with hygienic practices, re-infections can be cut to a minimum. At present, there is no known chemical treatment for blackleg but a systemic bactericide active against the blackleg bacterium would undoubtedly be a great help in maintaining health.

Nevertheless, it seems likely that, in the long term, the stem cutting procedure will make a great impact on tuber health in Scotland and contribute significantly towards a reduction in the cost of potato production throughout the United Kingdom.

References

GRAHAM, D. C. and HARPER, P. C. (1967) Potato Blackleg and Tuber Soft Rot. Scottish Agric. 46, 68-74.

HIDE, G. A., HIRST, J. M. and GRIFFITH, R. L. (1969) Control of Potato Tuber Diseases with Systemic Fungicides. Proc. 5th Br. Insectic. and Fungic. Conf. 2, 310-314.

STEWART, D. J. (1962) A Selective-Diagnostic Medium for the Isolation of Pectinolytic Organisms in the Enterobacteriaceae. Nature, Lond. 195, 1023.

Proceedings of the Third International Conference on Plant Pathogenic Bacteria, Wageningen, 14-21 April 1971

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Proceedings of the Third International Conference on Plant Pathogenic Bacteria

The Third International Conference on Plant Pathogenic Bacteria was organized for those scientists who are working with plant pathogenic bacteria. Their interests include microbiology, biochemistry, physiology, pathology or taxonomy. Consequently the programme of this meeting included a variety of subjects.

One of the aims of the conference was to provide general information on current work, so that participants working in one discipline could learn of recent development in others. To achieve this, some sessions were devoted to reviews, combined in symposia on special topics prepared by specialists.

In addition, papers were read dealing with results of personal research and giving up-to-date information on current work. Besides there was the opportunity to present short papers on problems or negative results met with during investigations. These contributions were then discussed, either in a plenary session, or in small groups of people particularly interested in the subject. These discussions were intended to stimulate the exchange of knowledge and ideas but, as they were often long and detailed, it was impossible to summarize them. Therefore in this book only the papers that were presented are published.

The reader will also find in these Proceedings long review articles, reports of results and short contributions dealing only with the description of problems.

All together, the contents will give a good idea of the main subjects with which the phytobacteriologists are concerned at present.

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Contents

Identification of soft rot coliform bacteria

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Abstract

The soft rot coliform bacteria, members of the genus *Erwinia*, are usually associated with soft rot diseases of plants. They are now generally considered to form a single species, *E. carotovora*, with several varieties *(atroseptica, chrysanthemi, rhapontici* and *cypripedii)*.

To study the phenotypic characters of all supposed members of this genus, 128 cultures have been examined in 90 tests. Seven tests have shown to be sufficiently consistent to be used for distinguishing between *E. carotovora* and the varieties *atroseptica* and *chrysanthemi*, and these, together with tests for fermentative metabolism of glucose, rotting of potato slices and slide agglutination with antiserum prepared against var. *atroseptica* can be used for routine identification. Mainly because only 3 isolates of var. *rhapontici* and var. *cypripedii* were available, they are not considered, though their reactions in the same tests are given for comparison.

The identity of pectolytic *Erwinia* spp. not belonging to the soft rot group, and certain 'atypical' *Erwinia* spp. is discussed.

Introduction

The soft rot coliform bacteria are gram-negative, peritrichously flagellated rods, having the characters of the Enterobacteriaceae, and which are usually, though not exclusively, associated with soft rot diseases of plants. In the 7th edition of Bergey's Manual these bacteria are all included in the genus *Erwinia*, but this genus contains at least two groups of plant pathogens which differ in pathogenicity: the soft rot coliform bacteria, and those causing necrosis and wilts typified by *E. amylovora*. Waldee (1945) suggested that these two groups should be distinguished at generic level, proposing that the name *Erwinia* be used only for the organisms associated with necrosis and wilts and establishing a new genus, *Pectobacterium*, for the organisms associated with soft rots. From further studies of phenotypic characters, Graham (1964) concluded that separation into these two genera is justified, but this view has not gained general acceptance. There are various reasons for this; for instance, results of DNA base composition analyses have emphasised the close relationship of the two groups, organisms which appear to be intermediate in character have been found, and organisms occur on plants (and in other habitats) which are not plant pathogens but plainly are closely related to them. The latter organisms belong to the species *E. herbicola*.

With regard to the soft rot group, the 7th edition of Bergey's Manual describes five species: *E. carotovora*, *E. atroseptica*, *E. aroideae*, *E. chrysanthemi* and *E. rhapontici*. However, more recent studies have emphasised the close similarity of all five species and, as Graham (1964) and Dye (1969) have concluded, the results indicate that there should be only one species: *E. carotovora*. Within this species, Graham *(op. cit.)* recognised three varieties: var. *aroideae*, var. *atroseptica* and var. *chrysanthemi*, mainly because this concept was useful for the plant pathologist. Since then, however, a wider range of isolates from many plants from various parts of the world has been studied by Dye (1969) and Graham (unpublished). Dye agrees that there should be only one species, but he no longer recognises anaerogenic strains (var. *aroideae*) as a separate taxon and he incorporates them in *E. carotovora*. This is justified because gas production is a variable character and failure to produce gas cannot be correlated with other characters. In his system, Dye includes four varieties of *E. carotovora*, namely var. *atroseptica* (the potato blackleg organism), var. *chrysanthemi*, var. *rhapontici* and var. *cypripedii*. It is likely that this classification will become generally accepted.

Looking for tests suitable for use in identification

About four years ago, I decided to examine a large number of cultures representing all supposed members of the genus *Erwinia*, with a view to perform a numerical taxonomic analysis, though the study was motivated by ecological and epidemiological rather than taxonomic considerations (one main purpose being to try to find tests which could distinguish the potato blackleg organism from all other soft rot coliforms). This study is not yet finished, but work on testing the carotovora organisms has been completed together with a preliminary sorting to pick out characters which could be used for routine identification. In all, 128 cultures were examined in 90 tests. The organisms were isolated from 36 different host plants, as well as one soil and one water sample, in 17 different countries.

The three cypripedii isolates did not liquefy pectate gel or cause soft rot of potato tubers, onion, or cucumber slices and therefore did not appear to be pectolytic. The three rhapontici isolates did not liquefy pectate gel, but slowly rotted potato slices and as distinct from all other cultures, produced a diffusible reddish pigment on several media. Because var. *cypripedii* is not associated with soft rot of plants, and var. *rhapontici* is found only on rhubarb, together with the fact that only three isolates of each variety were available, neither was considered along with *E. carotovora* and vars *atroseptica* and *chrysanthemi*. Some of their properties are given in Table 1.

The data on the remaining 122 isolates were first sorted by picking out those tests in which 95% or more of the organisms gave either positive or negative reaction:

List of tests in which the soft rot coliforms gave 95% or more positive or negative reactions

Positive: motility at 26°C; fermentative metabolism of glucose; acid from glucose, salicin, xylose, sucrose, arabinose, mannitol, glycerol, mannose, ribose, cellobiose; H_2S production; gelatin liquefaction; pectate gel liquefaction; utilization of citrate, acetate, mucate, galac-

Table 1. Reactions of *Erwinia carotovora* var. *rhapontici* and var. *cypripedii* in the tests listed in Tables 2 and 3.

Test	*E.c.* var. *rhapontici*	*E.c.* var. *cypripedii*
O/F test (glucose)	F	F
Potatoslice rotted	+	−
Acid from lactose	+	− or ×
maltose	+	+
trehalose	+	+
α-methylglucoside	+	−
Indole	−	−
Growth in 5% NaCl	+	+
Lecithinase	−	−
Phosphatase	+	+
Reducing substances from sucrose	+	−
Sensitivity to erythromycin	S	S
Agglutination with atroseptica antiserum	−	−

Notation as in Tables 3 and 4.

turonate; reduction of nitrate; potato slice rotted; catalase; presence of haem; ONPG; sensitivity to chloramphenicol, kanamycin, neomycin, nitrofurantoin, polymyxin, streptomycin, tetracycline.

Negative: Gram at 48 h; acid from erythritol, adonitol, dulcitol; occurrence of symplasmata, biconvex bodies, hydrolysis of starch, arbutin, hippurate; gluconate; oxidase; growth in 10% NaCl; haemolysis; yellow non-diffusible pigment; red non-diffusible pigment; blue pigment on CYC agar; ornithine and lysine decarboxylase; phenylalanine deaminase; urease; DNase; utilization of tartrate; sulphatase, diffusible pink pigment; yellow diffusible pigment on potato; sensitivity to fucidin; methicillin, novobiocin, oleandomycin.

Using the results of the remaining tests, the organisms were sorted into three groups representing *E. carotovora* and vars *atroseptica* and *chysanthemi*. For var. *atroseptica* this was done by comparing reactions of authentic strains (checked for pathogenicity on potato stems) with all other isolates and picking out those which gave very similar reactions. As expected, most of these organisms were isolated from potato; those from other sources were tested for pathogenicity on potato stems of the susceptible cultivar Majestic grown in controlled environment chambers at 65 °F. Those not causing blackleg were classified as *E. carotovora* (details of pathogenicity tests are in Table 2). The var. *chrysanthemi* was selected by comparing the properties of authentic cultures of var. *chrysanthemi* with those of the rest of the organisms; no pathogenicity tests were made. The cultures whose characters did not correspond with those of var. *atroseptica* or var. *chrysanthemi* were regarded as *E. carotovora*.

In all, 30 tests were made, 10 of which gave reasonably consistent positive of negative reactions within each of the three groups (80% or more of the isolates giving positive or negative results). These tests are given in Table 3. The other 20 tests (see page 277) gave variable results within each group (21%–79% of cultures positive).

Table 2. Results of pathogenicity tests on potato stems with organisms having the morphological, physiological and biochemical properties of *Erwinia carotovora* var. *atroseptica*, isolated from sources other than potato.

Code number	Host	Origin	Blackleg production	Remarks
J3	Chinese cabbage	Japan	−	agglutinates with atroseptica antiserum
J9	carrot	Japan	−	no agglutination with atroseptica antiserum
C399	tomato	UK	+	agglutinates with atroseptica antiserum
C403	tomato	UK	+	no agglutination with atroseptica antiserum
C407	cauliflower	UK	+	no agglutination with atroseptica antiserum
GEJ50	water from water-cress bed	UK	+	agglutinates with atroseptica antiserum
G107	Iris rhizome	UK	+	agglutinates with atroseptica antiserum
G103	tomato	UK	+	agglutinates with atroseptica antiserum

All organisms producing blackleg were considered to be var. *atroseptica*.

Table 3. Tests giving 80% or more positive or negative reactions, which differ between *Erwinia carotovora* and its varieties *atroseptica* and *chrysanthemi*.

Test	*E. carotovora* (60 isolates)	var. *atroseptica* (39 isolates)	var. *chrysanthemi* (623 isolates)
Acid from lactose	+ (96)	+ (100)	− or × (100)
maltose	− (80)	+ (87)	− (100)
trehalose	+ (94)	+ (98)	− (100)
α-methylglucoside	− (85)	+ (94)	− (100)
Indole	− (89)	− (100)	+ (100)
Growth in 5% NaCl	+ (100)	+ (94)	− (100)
Lecithinase	− (100)	− (100)	+ (92)
Phosphatase	− (100)	− (100)	+ (100)
Reducing substances from sucrose	− (90)	+ (98)	− (96)
Sensitivity to erythromycin	R (90)	R (94)	S (100)

Notation: lactose + = acid produced in 2 days in 1% lactose + 1% peptone water + bromothymol blue; lactose − or × = either no acid produced in 14 days or acid produced after at least 5 days incubation; maltose, trehalose and α-methylglucoside + = acid produced in 1% peptone + 1% sugar + bromothymol blue within 14 days; indole test + = positive in peptone water after 2 days incubation using Ehrlich's reagent; growth in 5% NaCl peptone water + = positive after 7 days; lecithinase + = opaque zone around colonies on egg yolk agar after 7 days; phosphatase + = pink colour of and around colonies grown for 48 h on 0.05% w/v sodium phenolphthalein diphosphate agar and treated with gaseous ammonia; reducing substances from sucrose + = positive in 4% sucrose peptone water (shake culture) in 48 h tested with Benedict's reagent; sensitivity to erythromycin: R = organism shows no inhibition zone around colonies using Multodisk test discs (potency 50 μg), S = clear inhibition zone visible. Incubation temperature for all tests 25 °C. Figures in brackets are percentages of cultures giving the respective positive and negative reactions.

List of tests giving variable results within E. carotovora *and vars* atroseptica *and* chrysanthemi

Gas from glucose; acid from raffinose, inositol, sorbitol, inulin, on ethanol agar; MR; VP; liquefaction of carboxymethyl cellulose gel; utilization of malonate; hydrolysis of tributyrin, casein; mucoid growth on 5% sucrose agar; growth at 37°C; arginine dihydrolase; KCN; sensitivity to ampicillin, colomycin, penicillin.

On the basis of these tests, the variety *chrysanthemi* includes *E. carotovora* f.sp. *parthenii*, *Pectobacterium parthenii* var. *dianthicola*, *E. carotovora* f.sp. *zeae*, *Pectobacterium carotovorum* var. *graminarum*, *E. dieffenbachiae*, *E. cytolytica* (NCPPB 1385), the organism isolated from pineapple rot in Malaya (NCPPB 551), and an organism received from France as *E. betivora* isolated from soft root of beet. Dye (1969) says that the majority of the strains of var. *chrysanthemi* produce a blue insoluble pigment (indigoindine) on glucose-yeastrel chalk agar, but I have found this to be inconsistent with many isolates, though it is distinctive of this variety when it occurs. Sometimes it is produced in the rotted tissue of potato slices, especially with the cytolytica and betivora isolates, and one of the f.sp. *zeae* isolates.

Routine identification at the East Craigs laboratory

For routine identification of soft root bacteria from plant tissue, material is plated on MacConkey-pectate gel medium (Stewart, 1962), and also on nutrient agar or King medium B when isolations are made from sources other than potato. Colonies are picked and transferred to nutrient agar slopes and the organisms are routinely put through the tests indicated in Table 3, together with those for acid from lactose and trehalose, growth in 5% NaCl reducing substances from sucrose, lecithinase, phosphatase and sensitivity to erythromycin given in Table 3. The three tests mentioned in Table 4 giving less than 90% positive or negative reactions within each group are not used for routine identification.

Agglutination with atroseptica antiserum is not specific for the blackleg organism, but is a very useful and rapid test for its presence in diseased tissue, and also for a quick check on colonies on isolation plates (good reactions can be obtained with

Table 4. Additional identification tests used for soft rot coliform bacteria newly isolated from plant tissue.

	E. carotovora	var. *atroseptica*	var. *chrysanthemi*
O/F test (glucose)	F	F	F
Potato slice rotted	+	+	+
Agglutination with atroseptica antiserum	d	+	−

Notation: F = fermentative metabolism of glucose. Agglutination tests done on slides; d = different isolates give consistently different reactions.

colonies on MacConkey-pectate gel plates). Two isolates with all the morphological, physiological and biochemical characters of var. *atroseptica*, and which caused potato blackleg on inoculation into potato stems, did not give slide agglutination reactions with atroseptica antiserum. One was isolated from a stem rot of tomato, the other from a rotted cauliflower head, both in Eastern England. This is in contrast to about 250 isolates of atroseptica from potato (obtained mainly in Scotland), which all gave good agglutination reactions. It is noteworthy that one culture, identified as var. *chrysanthemi*, was isolated from potato blackleg in Brazil. It is the only case in my experience where this organism has been found associated with blackleg, but so far I have not checked its pathogenicity on potato stems. All chrysanthemi isolates gave slide agglutination reactions with antiserum prepared against NCPPB 1385, whereas no agglutination occurred with atroseptica isolates. At least some carotovora isolates reacted with chrysanthemi antiserum, but the number of tests has as yet been insufficient to see if reactions occur so frequently as to make the test of no value in routine identification.

Pectolytic Erwinia species not included in the carotovora group

E. salicis causes watermark disease of willow, rots potato slices, and forms depressions in pectate gel without actually liquefying it (the rotted potato tissue becomes yellow, the pigment is diffusible).

E. rubrifaciens causes a disease of walnut, produces depressions around colonies on pectate gel, but does not rot potato slices.

E. quercina, the cause of a disease of acorns, gives a slight soft rot of potato slices but is not pectolytic on pectate gel (the effect on potato is the production of a water-soaked area in which the tissue is slightly softened; it is not a typical soft rot).

Erwinia salicis and *E. rubrifaciens* resemble *E. amylovora* in most respects, but *E. quercina* is, on the whole, most like *E. carotovora*. None of these organisms has ever been isolated from other hosts.

Identity of some atypical Erwinia species

E. dissolvens NCPPB 1862, and the organism from Kona coffee cherries described by Frank, Lum & Dela Cruz (1965) as *E. dissolvens*, have the characters of *Klebsiella aerogenes* and do not rot potato slices or liquefy pectate gel. *E. dissolvens* NCPPB 1850 is non-motile but otherwise has the characters of *Enterobacter cloacae*.

E. nimipressuralis NCPPB 440, the cause of 'wet wood' of elm in the USA, was received from Professor Burkholder as an authentic culture. It does not rot potato slices or liquefy pectate gel and has the characters of *Enterobacter cloacae*. An organism from elm in the UK, received from Dr J. Rishbeth, produces depressions around colonies on pectate gel plates but does not rot potato slices. It has the characters of an *Erwinia*, but so far I have not been able to identify it (though it resembles the carotovora group more closely than the amylovora group).

E. carnegieana NCPPB 439, said to be the cause of a disease of the giant cactus, was also received from Professor Burkholder as an authentic culture. It does not liquefy pectate gel or rot potato slices, and I am uncertain on its identity, though it may be an *Enterobacter*. *E. carnegieana* NCPPB 671 and 672, more recently isolated from the giant cactus, are *E. carotovora*.

References

Dye, D. W., 1969. A taxonomic study of the genus *Erwinia*. II. The 'carotovora' group. N.Z. J. Sci. 12: 81–97.

Frank, H. A., N. A. Lum & A. S. Dela Cruz, 1965. Bacteria responsible for mucilage-layer decomposition in Kona coffee cherries. Appl. Microbiol. 13: 201–207.

Graham, D. C., 1964. Taxonomy of the soft rot coliform bacteria. A. Rev. Phytopath. 2: 13–42.

Stewart, F. J., 1962. A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. Nature, Lond. 195: 1023.

Discussion group: Physiology

Discussion group: Miscellaneous Papers

Discussion group: Serology

Symposium II: Plant Reaction to Infection

Discussion group: Pathogenicity Tests

Symposium III: Biochemistry of Tumor Development

Discussion group: Taxonomy

Discussion group: Virulence

Symposium IV: Mycoplasmas

Symposium V: Enzymes and Toxins

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8. PLENARY SESSION ON THE STORAGE ENVIRONMENT

STORAGE REQUIREMENTS FOR THE CONTROL OF TUBER DISEASES AND BLEMISHES

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At the risk of stating the obvious I think we should begin by reminding ourselves that potato tubers are storage and perennating organs of a species of Solanum, and tubers are not designed by nature to be lifted, separated and stored in the mass. We use the tubers for food or as seed for propagation and as they are living organs and must be kept alive for several months, even the unimaginative appreciate that certain storage conditions must be met if tubers are not to deteriorate by premature sprouting, water loss and as a result of microbial activity. It is obvious that, to achieve good keeping, tubers need to be kept cool and dry. There are many storage methods which provide these conditions, and, in Scotland in past years, the most important was the pit or clamp, though this has given way largely to storage in unventilated sheds, ventilated sheds with or without forced draught systems, and pallet boxes.

In practice, the environmental conditions need to be reasonably closely defined because tuber storage is complicated by many factors, one of the most important being the problem of biodeterioration caused by pathogenic fungi and bacteria. The most important storage pathogens in Scotland at the moment are gangrene (caused by *Phoma exigua* var. *foveata*), soft rot (associated largely with soft rot coliform bacteria of the genus *Erwinia*), skin spot (caused by *Oospora pustulans*), late blight (*Phytophthora infestans*), and dry rot (caused mainly by *Fusarium solani* var. *caeruleum* in Scotland). Diseases of less importance include silver scurf (caused by *Helminthosporium solani*) and pink rot (*Phytophthora erythroseptica*), though silver scurf can cause a severe blemish and make washed tubers unattractive to the buyer, as well as allowing tubers to lose moisture, causing flaccidity.

Soft rot, late blight, dry rot, pink rot and gangrene are rots of the tuber flesh that can progressively destroy the tuber in store, whereas skin spot and silver scurf are superficial infections, the special importance of skin spot being its destruction of tuber eyes which can effect sprouting of seed tubers.

Gangrene, soft rot, and dry rot are associated with tuber damage to a greater or lesser degree. Gangrene seems to start when the fungus is introduced into the flesh when the tuber is damaged, either from outside the tuber or from latent infection already in the periderm; or, of course, from both sources. Wound infection may occur at lifting, grading, transport or at any time of handling, but symptoms seem to follow lifting injury quite frequently, and crush wounds are more likely to become diseased than clean cuts. Though resistance to spread of the fungus in the flesh generally declines as the period of storage lengthens, it is noteworthy that immature tubers are very susceptible, suggesting that factors which retard maturation, such as application of fertilizers at high rates, result in greater susceptibility.

Soft rot coliform bacteria apparently cannot penetrate periderm directly, but enter tubers through wounds and lenticels and also via the stolon in plants affected by blackleg disease. The bacteria which infect daughter tubers are released into the soil from the decomposing mother tubers, and so widespread is infection that almost all ordinary

commercial potato stocks in Scotland carry these organisms. The blackleg organism, *E. carotovora* var. *atroseptica* is the most important cause of soft rot in Scotland, whereas *E. carotovora* plays a secondary role and is not associated with blackleg symptoms in the growing crop. Other organisms which can be involved are soft rot pseudomonads and *Bacillus* spp. In experiments carried out in my laboratory it was found that tuber susceptibility to *Erwinia* soft rot increased as the storage period lengthened.

Dry rot fungi are wound parasites that persist in soil and enter damaged areas which may occur at harvest, on grading, during transport and handling. Experience shows that areas damaged at lifting are less likely to become affected by dry rot than by gangrene, but it is noteworthy that towards the end of the storage period it is not uncommon to find dry rot spreading from gangrene lesions. Most disease develops after grading, and hence dry rot is often thought of primarily as a seed tuber disease, as the ware is usually disposed of quickly. Tuber resistance is important in disease development; some cultivars are especially susceptible and, in general, resistance declines during storage but despite many studies, the nature of the mechanism of resistance to infection is still not clear.

Late blight, pink rot, skin spot and silver scurf are diseases which are not especially associated with mechanical damage. Blight infection commonly occurs when spores are washed down by rain from infected haulm, and penetration takes place through eyes, lenticels, and tiny wounds though not through intact periderm. Pink rot, which occurs sporadically, has not been extensively studied. Infection characteristically takes place through the stolon from plants affected during growth.

Skin spot is a skin disease, the symptoms becoming obvious only after several months storage as small pustules on the skin surface and in the eyes. Penetration is generally limited to roughly 12 layers of surface cells and although it does take place through lenticels, eyes and superficial wounds, apparently undamaged areas of skin are often affected. Cultivars vary considerably in susceptibility to skin spot.

Silver scurf is also a skin disease, the fungus entering through cells of the periderm or lenticels with subsequent disintegration of the periderm. Lifting of the outer layers occurs, resulting in the silvery grey appearance of affected skin.

Except for bacterial soft rot there is little evidence that spread of disease takes place by tuber contact and tubers are either infected or contaminated before they are brought into store. The visible manifestation of this infection then depends on many factors affecting the interaction of host with pathogen, so that in some circumstances the pathogenic mechanisms of fungus or bacterium overcome the defences of the host, while in others the host succeeds in containing the pathogen. Host biochemistry is involved in defence reactions, for example the formation of specific antimicrobial substances such as rishitin and phytuberin in tubers in response to infection by the late blight and dry rot fungi, and oxidation of chlorogenic acid to its quinone, a substance which has strong antibacterial properties.

However, in practice, the physical environment plays a major role in disease development, especially temperature and humidity, since these two factors influence fungal and bacterial growth, enzyme activity, and the laying down of a cork barrier by the host to prevent invasion of healthy tissue. Studies on this aspect have been made by a number of workers in recent years and I shall discuss some of the results briefly. Gangrene tends to develop best at low temperatures. Experiments carried out by the Potato Marketing Board showed that in one case 37·5% of tubers stored at 2° developed gangrene, while at 10° only 5·1% became affected. In another trial 14·1% were affected at 2° and 0·3% at 7°. In an experiment done by Dr. A. E. W. Boyd at the East of Scotland College of Agriculture where tubers were inoculated with gangrene fungus and held at different temperatures in high humidity the cultivar Redskin showed 80% infection after 11 weeks at 4° plus 8 weeks at 16°, and only 15% at 16° after seven weeks storage; whereas, using

cultivar Catriona, 43% of tubers became diseased after 11 weeks at 4° plus eight weeks at 16°, while none were affected at 16° after seven weeks.

Dry rot when it is caused by *F. solani* var. *caeruleum*, is a disease which appears to be favoured by lower temperatures in the early period of storage, which increases the susceptibility of the host, followed later by higher temperatures, which probably stimulate growth of the fungus. Interestingly enough tests show *F. solani* var. *caeruleum* requires higher temperatures for good growth than the gangrene fungus. Twenty-three per cent of artificially inoculated tubers of Redskin kept at 4° for 11 weeks and then at 16° for eight weeks developed dry rot, whereas none were affected at 16° after seven weeks. With cv. Catriona, which is very susceptible, the results were 95% infection in tubers held at 4° for 11 weeks plus eight weeks at 16°, whereas only 60% of tubers were affected after seven weeks at 16°. (The tubers could be held at 16°C only for a comparatively short time because of excessive sprouting and loss of turgor.)

Soft rot is a disease favoured by high temperatures. In an experiment where tubers of cv. Pentland Crown were inoculated with standardised suspensions of *E. carotovora* var. *atroseptica*, and held at high humidity for seven days the total wet weight of rotted tissue taken from inoculation sites on 12 tubers was 3 g at 4°, 80 g at 12° and 172 g) at 18°. The progress of infection is arrested under relatively low humidity (c. 80% R.H.) and there is strong evidence that rotting does not spread from primary foci unless there is a film of water on tubers.

The skin diseases skin spot and silver scurf well illustrate the effect of the interaction of humidity and temperature on disease development. At low humidity, the incidence of skin spot is low over the temperature range 2–12° while at high humidity, disease incidence is greatest at 2° and least at 12°. With silver scurf, the incidence is also low over the temperature range 2–12° at low humidity, but at high humidity, the surface area of skin affected is greatest at 12° and lowest at 2° – the opposite of skin spot.

Regarding the rot diseases of tubers associated with damage, probably the most significant defence reaction of healthy tissue is the laying down of a cork barrier which prevents ingress of the pathogen. The optimum environmental conditions under which the barrier forms and the damaged area heals is around 18° at high humidity. In practice, roughly the right conditions can be achieved by restricting ventilation as soon as possible after loading, particularly in bulk stores. Natural heating and sweating then raise the temperature and humidity, and it is usual to maintain these conditions for about 10 days, when healing is more or less complete. This is the so-called 'curing' period. A major difficulty arising from this procedure is that curing conditions are those which encourage the development of bacterial soft rot, so that the temperature must be reduced as quickly as possible after curing is finished. Additionally, if the crop has quantities of soil associated with it, or has been harvested damp, or contains many tubers affected by late blight or soft rot, curing cannot be carried out safely. Curing generally does not prevent development of soft rot because bacterial attack and disintegration of host cells is so rapid that tubers are unable to form cork barriers fast enough to contain the invasion. This treatment also has limitations in healing wounds and thus preventing the later development of rots, especially at sites where tissue has been crushed. For instance, in one experiment, gangrene developed on 73% of crush wounds where tubers were not given a curing period, but 50% of crush wounds still developed gangrene even after the tubers were cured. Better results were obtained when tubers with clean cuts were cured but crush wounds are common under commercial conditions.

So far we have considered the effect of physical environmental factors on disease but certain chemical factors can play a role, and in particular, the chemical composition of the storage atmosphere. Generally speaking, however, this gives no trouble, provided good ventilation through the tubers is achieved. If ventilation is imperfect or obstructed,

reduced oxygen and increased CO_2 concentrations can result through tuber respiration, and experience shows that, in turn, bacterial soft rot can be enhanced.

What conclusions can we draw from the data we have examined? It seems to me that in countries where many different storage diseases occur, no general regime of storage treatment can be defined which will control all diseases satisfactorily. Indeed, I suggest we should not attempt to control diseases in this way. At best I believe the data simply help to indicate undesirable environmental conditions which growers and merchants should try to avoid if possible. In the end, we must recognise the main aims of good storage are to prevent weight loss, check sprouting and to provide material with the correct chemical composition for processing, as well as to minimise disease, but the conditions necessary to prevent moisture loss and sprouting are conducive to several diseases. The simple fact is, if something bad is put into store, something good cannot be expected to come out.

But how then are diseases to be controlled? There are several ways of tackling this problem, one of the most important being the reduction of tuber damage, and I think we will all agree there is much room for improvement. In addition, methods must be adopted to avoid infection of tubers before the storage stage is reached. Recent research has emphasised that in the case of several tuber diseases including skin spot and *Erwinia* soft rot, the mother tuber is the main source from which the pathogens spread and infect the new tubers in the growing crop. Hence the stem-cutting method of propagation combined with good farm hygiene and husbandry methods to avoid reinfection and supplemented by chemical disinfection to deal with re-contamination, can achieve very good control on seed. These benefits will eventually pass on to the ware crop. The disinfectants used are likely to be systemic fungicides applied as powders to the seed, or antifungal fumigants introduced into specially designed fumigation chambers. At present blackleg and bacterial soft rot caused by *Erwinia* spp. can be eliminated only by propagation using tested stem cuttings, as there is as yet no chemical treatment capable of destroying bacteria within plant tissue. However, it is noteworthy that the Japanese firm of Takeda recently discovered a bactericide which acts systemically in plants, but it unfortunately proved to have too great a mammalian toxicity for general use. Nevertheless, I think we can look forward to some new developments in this direction, now that a systemic bactericidal molecule is known.

There is, of course, still the problem of those disease organisms that are soil borne, and to deal with these there is another general method of control I think we should be considering, namely altering the chemical environment by applying anti-microbial fumigants to tubers in store. Such substances could be introduced either as vapours or fogs. They probably could be used only in stores fitted with forced draught ventilation systems though I think it might be possible to use both sheds and bins without drastic design modifications. In a sense, the idea of using fumigants is not new because the use of tecnazene (TCNB) powder to control both dry rot and sprouting in store is a form of fumigation as it is the vapour given off from the solid that is the active phase. It is, however, noteworthy that tecnazene is now under suspicion of being carcinogenic, and this problem illustrates a key difficulty in using chemicals on staple foods like the potato. They must be of very low toxicity and must not leave too high a residue in tuber tissue if they are to be acceptable on ware nor should they be too toxic to handle. Nevertheless, I do not believe it is beyond our skill to find substances with these properties. Promising chemicals are already available for use on seed and might be acceptable on ware, the best so far being 2-aminobutane, which gives excellent control of both skin spot and gangrene and some control of silver scurf, though it has no activity against dry rot, soft rot or blight. In a large scale co-operative experiment, using a bin, a very successful fumigation of 38 tons of tubers was done in October 1970, by workers from my station and colleagues at the Edinburgh School of Agriculture. With the cv. King Edward no

gangrene was found in fumigated tubers held for about five months at around 4-6° either by outside air cooling or refrigeration with recirculation, whereas with unfumigated tubers held at the same temperature by outside air cooling, 41 % developed gangrene and of those under refrigeration with recirculation, 21 % developed gangrene. Both fumigated and unfumigated material had been given a curing period.

We are now investigating other substances such as cyclohexylamine and dibromotetrachloroethane as fumigants and dichlorophen as a fog. Dichlorophen is particularly interesting as it is said to control bacterial soft rot, though we have found it will not control gangrene or skin spot. You may also be interested to know a patent was taken out in the United States as long ago as 1958 for control of dry rot with dibromotetrachloroethane.

To conclude then, I suggest that while storage management techniques can never control storage diseases we can make some adjustments to the environment to minimise losses. The main ways of controlling diseases lie in production of healthy seed by integration of agronomic procedures and chemical treatments, and perhaps also chemically treating tubers in store. I believe the prospect of achieving very good results has never been brighter and we should concentrate our research and development effort along these lines.

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Use of 2-aminobutane as a fumigant for control of gangrene, skin spot and silver scurf diseases of potato tubers

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Zusammenfassung, Résumé p. 123

Summary

Fumigation of tubers with 2-aminobutane (sec-butylamine) gas at a dosage of 200 mg/kg in simply made fumigation chambers gave very good control of the diseases gangrene (caused by *Phoma exigua* var. *foveata*) and skin spot (*Oospora pustulans*) if treatment was done within 14 days of harvesting. Some control of silver scurf disease (*Helminthosporium solani*) was also obtained, but results were always poorer than with gangrene or skin spot. Fumigation did not control tuber blight (*Phytophthora infestans*), dry rot (*Fusarium solani* var. *caeruleum*) and did not kill the sclerotia of *Rhizoctonia solani*.

Since chemical analysis of treated peeled and boiled tubers, and crisps, granules and flakes made from treated tubers showed that they contained substantial residues of 2-aminobutane, the treatment can only be used on seed tubers. There were no significant residues in crops grown from treated tubers.

Introduction

The potato tuber can be attacked by many fungal and bacterial diseases during storage. At present one of the most troublesome tuber diseases in Scotland is gangrene, which is a rot of the tuber flesh caused by *Phoma exigua* var. *foveata*. Skin spot, caused by *Oospora pustulans*, is also important on certain susceptible varieties. It appears as small pustules on the tuber surface and more importantly, eyes may be killed, thus affecting sprouting. Silver scurf, caused by *Helminthosporium solani*, is a skin disease of less importance but it disfigures tubers and causes flaccidity by accelerating water loss through damaged skin.

Many attempts have been made to control these diseases chemically but until recently, the only successful method was dipping in a solution of an organo-mercury compound. This treatment has several disadvantages such as the toxic hazard associated with mercurials as well as the problem of drying tubers and has made only limited impact on the seed trade. However, the situation changed with the introduction of the systemic benzimidazole fungicides as dust and dip treatments (Hide et al., 1969) and fumigation of tubers with 2-aminobutane (sec-butylamine) (Graham and Hamilton, 1970). Graham and Hamilton (1970) drew attention to the basically simple idea of introducing gas into bulks of tubers, and outlined the fumigation process briefly. They gave some data on the degree of control of skin spot and gangrene both when

small quantities of tubers were fumigated at different dosages and when bulk fumigations were done in a specially designed chamber holding 5000 kg of tubers.

This paper describes the process of fumigation in more detail, briefly outlines the construction of a special cylindrical fumigation chamber and two chambers for bulk fumigations and discusses results of fumigating bulks of tubers at different dosages at various times after lifting to determine the degree of disease control. Residue levels in treated and processed tubers are also given.

Some properties of 2-aminobutane

2-Aminobutane is a colourless or pale amber liquid, boiling point 63 °C, and thus easily vaporised. It is inflammable (flash point −19.5 °C) and the lower explosive limit in air is 21–25 g/kg, but such concentrations are never reached when the chemical is used as a potato fumigant. 2-Aminobutane is an organic base which, having an asymmetric carbon, exists as optical isomers, and the commercially available products are racemic mixtures. The (−) enantiomorph is considerably more biologically active than the (+), both in preventing spore germination and in inhibiting mycelial growth (Eckert et al., 1970). 2-Aminobutane is stable, but corrosive to tin, aluminium, copper, and its alloys and some steels.

It is a moderately toxic substance (rat oral LD_{50}, 380 mg/kg) and experience has shown that hazards relating to application are primarily due to its alkalinity. Like ammonia, these effects are minimised by dilution (it is miscible with water) or as the basicity is neutralised (Anon., 1966). Certain precautions need to be taken when using this substance and an official 'Code of practice' for safe use of the chemical on potatoes has been published, which may be obtained from the authors.

The process of fumigation and chamber construction

The first small-scale fumigations with 2-aminobutane were done in a steel fumigation chamber designed for work with other gases. These tests showed that absorption of gaseous 2-aminobutane by tubers was too rapid for the ct (concentration × time) product method of expressing treatment to be practicable, and so the ratio weight of fumigant to weight of potatoes treated, expressed in mg/kg, was used instead. It was also found that rapid stirring or better still some system of forced air recirculation was necessary to obtain good distribution of the gas.

Because good disease control was achieved in the early fumigations at dosage rates of around 200 mg/kg a prototype fumigation chamber holding 5000 kg of potatoes was built to study the process of fumigation with larger bulks of tubers. The design was basically a gas-tight wooden box reinforced with a steel frame (Fig. 1). It was 3 m long, 2 m wide and 2.5 m high with double doors at one end, the potatoes being held in position by removable bulkheads. The floor was made of boards 25 cm in width with carefully adjusted spaces between them to provide an even air flow up through the bulk of potatoes. Between the floor holding the potatoes and the bottom of the

Fig. 1. Five-tonne box fumigation chamber, with fan, vaporiser and recirculation piping system.

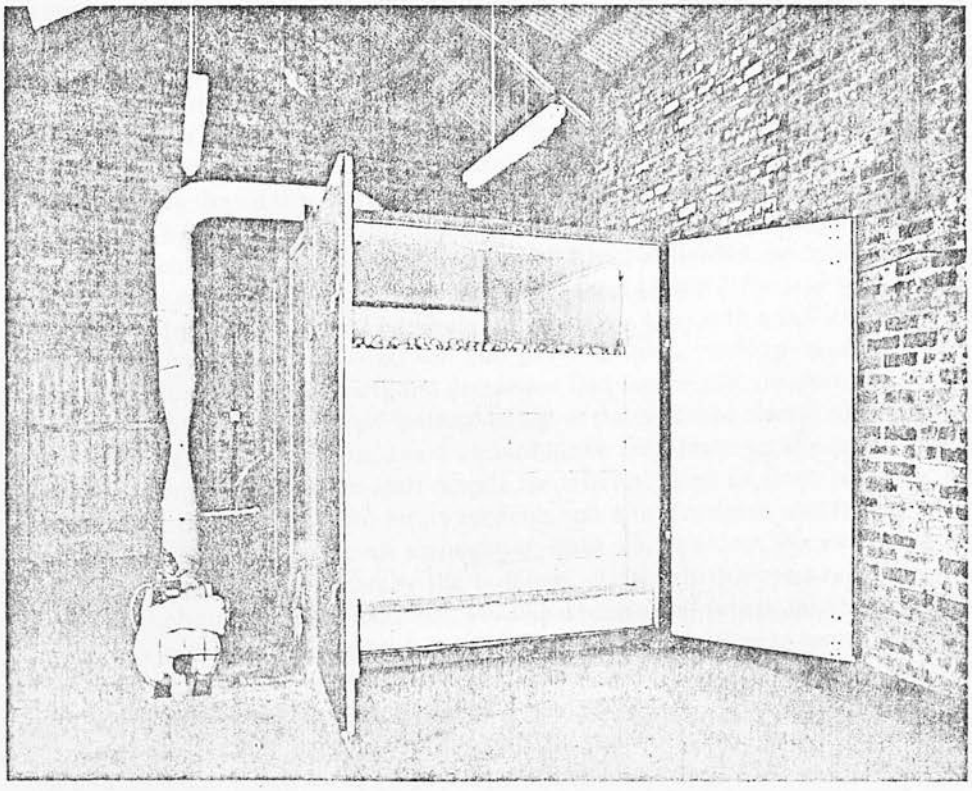

Abb. 1. Gaskammer, Kapazität fünf Tonnen, eingerichtet mit Gebläse, Verdampfer und für Gas-Innenkreislauf.
Fig. 1. Chambre de fumigation, capacité cinq tonnes avec ventilateur et vaporisateur, système de récirculation.

chamber there was a 20-cm high air space. All the internal surfaces were coated with a chemical-resistant paint. The air and gas was blown by a centrifugal fan via a box duct containing deflector baffles into the under-floor space, up through the potatoes, out through 0.3 m diameter trunking in the roof of the chamber and returned to the chamber via a vaporiser and the fan. The vaporiser consisted of a tank with a hot water jacket fitted with an immersion heater capable of raising the temperature to around the boiling point of 2-aminobutane, which was run into it through a small pipe in the lid. The vaporiser was built to ensure that the fumigant could be vaporised at any specified rate, otherwise vapourisation would have been dependent solely on the effect of the air stream. When the chamber was filled with potatoes, the height of the potatoes from bottom to top was about 1.5 m. Further details about the construction of this chamber can be obtained from the authors.

Because 2-aminobutane can be very rapidly absorbed by potatoes, most of the fumigant could be absorbed by the lower layers, resulting in inadequate distribution throughout the bulk. To overcome this difficulty it was decided to use a reasonably fast air flow of 28 m^3 per minute to try to blow the gas up through the bulk to the top before it was all absorbed. This flow of 5.6 $m^3\ min^{-1}\ t^{-1}$ was about 5 times the rate recommended for ventilation of potatoes (Anon., 1960). At first various rates of application of 2-aminobutane were also tried to see how this affected distribution.

Free gaseous 2-aminobutane measurements were taken during the fumigations. Samples were drawn from different positions inside the fumigation chamber and from within the bulk of potatoes through small bore polythene tubes which terminated at various positions and led outside the chamber. The 2-aminobutane concentration was measured by drawing gas through detector tubes designed for use with ammonia (ammonia tubes 5/a supplied by Draeger-Normalair Ltd) with a bellows attachment. The tubes were found to work well and gave the quick readings necessary because concentrations changed rapidly and demanded frequent measurements at many points. A concentration gradient from bottom to top of the bulk was always observed during the early part of fumigation, the 2-aminobutane only reaching the upper layers of potatoes some time after the start despite the relatively high air flow. However, it was found that at the end of two hours recirculation after fumigant was added, the concentration in the air was even throughout, although very low, because most of the fumigant had been absorbed by the potatoes. Although there was rapid absorption of 2-aminobutane by the potatoes, residue analyses of tubers taken from various positions in the bulk showed the distribution of 2-aminobutane to be reasonably even throughout using a dosage of 200 mg/kg, introduced in 30–40 min and the mixture of air and gas recirculated for a further 2 h (see Table 1).

To obtain more information on the physical process of fumigation, particularly the 2-aminobutane distribution on potatoes throughout a high bulk of tubers, a special chamber was made for this work. A steel cylinder of 0.1 m^2 cross sectional area and 3 m high was constructed, having clip-fastened gas-tight ports at the top, middle and bottom to allow easy access to the potatoes inside. It was fitted with a fan, vaporiser, flow meter and the necessary piping to complete a closed recirculatory system (Fig. 2). Samples of potatoes could be withdrawn within a minute while the fan and the fumigant application were stopped, so that samples could be taken both during and after the addition of the fumigant. More samples could also be taken at different stages of air recirculation. The cylinder held 175 kg of potatoes, compared with 5000 kg in the larger chamber. Thus the effects of different rates of application of fumigant, different air flow rates, and the best length of time for recirculation of air after all the fumigant had been introduced were studied realistically, but with relatively small amounts of potatoes. Much information was obtained, such as the fact that most of the 2-aminobutane was absorbed by the potatoes near the bottom of the cylinder during application of the fumigant. Much of this was desorbed then reabsorbed by the tubers above during the recirculation period so that the 2-aminobutane moved upwards through the column. However, for reasons which were not clear, these scaled-down

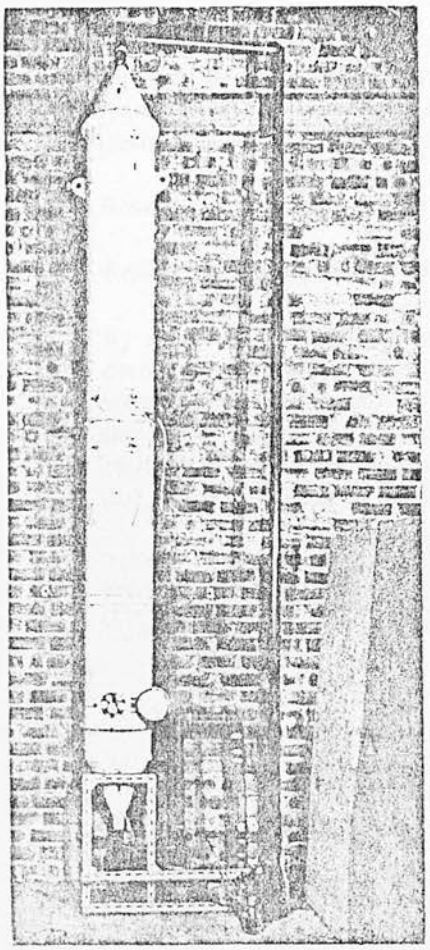

Fig. 2. Cylindrical fumigation chamber. Bottom port is open.

Abb. 2. Zylindrische Gaskammer. Die unterste Luke ist offen.
Fig. 2. Chambre de fumigation cylindrique. L'hublot le plus bas est ouvert.

treatments did not achieve such good distribution as in the 5-tonne treatments and the information obtained did not suggest improvements in the dosage and air flow or the recirculation time for 5-tonne fumigations, which had already been found empirically. The experiments did show, however, that it was possible to move 2-aminobutane from the bottom to the top of a column of potatoes 3 m high and that the absorption, desorption and reabsorption process took place irrespective of whether soil adhering to tubers was dry or moist.

Another fumigation chamber has been designed for the fumigation of boxed potatoes, based on the same principle as the 5-tonne prototype. It was constructed for fumigation of virus-tested stem cutting stocks of potatoes raised by the Department of Agriculture and Fisheries for Scotland. The fumigant-air mixture is blown up through 2 parallel ducts on which the boxes of potatoes are resting, out through

trunking in the roof, back through a fan and vaporiser before being recirculated through the boxes. This chamber was built with the help of the National Institute of Agricultural Engineering (Scottish Station). Details of construction may be obtained from the Director, NIAE, Scottish Field Station, Penicuik, Midlothian, Scotland.

Residues of 2-aminobutane in treated tubers

Residues of 2-aminobutane in relation to the distribution of gas in bulks of tubers

By analysis of 2-aminobutane residues in tubers it was possible to determine the evenness of 2-aminobutane distribution throughout bulks of tubers. The potatoes were analysed by the gas chromatographic method of Day et al. (1968), but omitting the use of the carbon tetrachloride wash. Considerable variation was found in the residues of individual potatoes and even of adjacent tubers. This was almost certainly due to the condition of the skin since higher residues were found in immature tubers

Table 1. Residues of 2-aminobutane in potatoes fumigated within 2 days of lifting in the 5-tonne prototype fumigation chamber.

Cultivar[1]	Dose[2] (mg/kg)	Sample position in chamber[3]	Residue[4] (mg/kg)
Redskin 1	200	top[5]	194
		bottom[6]	182
Majestic 1	200	top	103
		bottom	113
Redskin 2	200	top	92
		bottom	160
King Edward 1	200	top	92
		bottom	90
King Edward 2	50	top	12
		middle[7]	39
		bottom	32
Redskin 3	50	top	2
		middle	11
		bottom	23

[1] *Sorte – Variété*
[2] *Dosis – Dose*
[3] *Lage des Musters in der Kammer – Position de l'échantillon prélevé dans la chambre*
[4] *Rückstand – Résidu*
[5] *Oben – Sommet*
[6] *Unten – Base*
[7] *Mitte – Milieu*

Tabelle 1. Rückstände von 2-Aminobutan in Kartoffeln, die innerhalb von 2 Tagen nach der Ernte in der 5-Tonnen-Prototype-Gaskammer begast wurden.
Tableau 1. Résidu de 2-aminobutane dans des pommes de terre traitées par fumigation dans les 2 jours qui suivent l'arrachage, dans un prototype de chambre à fumigation de 5 tonnes.

and in others where the skin was damaged. To reduce this variation, sound quarters from each of four tubers of fairly uniform seed size were taken for each analysis.

Typical residues in potatoes fumigated in the 5-tonne fumigation chamber where the dose was introduced in 30–40 min and then recirculation done for a further 2 h are shown in Table 1. As mentioned above, good distribution with a dosage of 200 mg/kg was achieved, but at 50 mg/kg dosage most of the residue was confined to the lower tubers indicating this dose to be insufficient for equal distribution to occur.

There was some reduction in residue when potatoes were stored under well ventilated conditions, such as in open trays. However, the variation between individual tubers made it difficult to establish this with certainty, and substantial residues of 2-aminobutane were still present after many months storage.

Residues of 2-aminobutane remaining after peeling, cooking and processing

Residues of 2-aminobutane were determined to see if fumigation could be applied to ware (table) potatoes, since it was possible that residues might not remain after peeling, cooking or processing. Tubers given a dose of 200 mg/kg were used in these experiments.

Peeling

The fumigated potatoes were peeled with an ordinary hand peeler which removed

Table 2. Residues of 2-aminobutane in the peel and flesh of fumigated potatoes.

Cultivar[1]	Residue in flesh[2] (mg/kg)	Residue in peel[3] (mg/kg)	Calculated residue in whole tuber[4] (mg/kg)	Residue removed by peeling[5] (%)
Redskin 1	8	193	28	73
Majestic	13	294	48	75
Redskin 2	24	374	53	58
King Edward 1	28	508	66	60
King Edward 2	63	688	130	57
Redskin 3	16	365	45	66
King Edward 3	33	244	55	44
King Edward 4	22	192	40	50
Pentland Crown 1	24	471	62	64
Pentland Crown 2	23	420	56	70

[1] *Sorte – Variété*
[2] *Rückstand im Fleisch – Résidu dans la chair*
[3] *Rückstand in der Schale – Résidu dans la peau*
[4] *Berechneter Rückstand in der ganzen Knolle – Résidu calculé dans le tubercule entier*
[5] *Rückstand, entfernt durch das Schälen – Résidu éliminé par l'épluchage*

Tabelle 2. Rückstände von 2-Aminobutan in der Schale und im Fleisch der begasten Kartoffeln.
Tableau 2. Résidu de 2-aminobutane dans la peau et la chair de tubercules soumis à la fumigation.

about 10–12% by weight of the tuber. The peelings and flesh were analysed separately. The results shown in Table 2 indicate that about 60–70% of the residue was in the peel, so that significant amounts had passed into the flesh.

Cooking – boiling

Fumigated potatoes were peeled and halved and one half cooked by boiling in salted water and analysed. The other half was analysed to determine the residue level before cooking and in some cases the water in which the potatoes were cooked was also analysed. The results shown in Table 3 indicate that about 35% of the 2-aminobutane was lost during boiling. Some of this could be found in the cooking water. No allowance was made for the loss in weight of the potatoes during cooking, which can be as much as 30%.

Cooking – crisping

Fumigated potatoes were peeled and crisped under conditions used by commercial

Table 3. Effect of cooking on residues of 2-aminobutane in fumigated potatoes.

Treatment and cultivar[1]	Residue in uncooked flesh[2] (mg/kg)	Residue in cooked flesh[3] (mg/kg)	Residue removed by cooking[4] (%)
Cooking by boiling[5]			
Redskin 1	18.8	10.3	45
King Edward 1	42.4	26.1	39
Majestic 1	53.0	37.0	30
Majestic 2	20.0	15.3	24
King Edward	10.0	5.9	41
Pentland Crown 1	24.0	15.1	37
Pentland Crown 2	14.1	8.4	40
Cooking by crisping[6]			
King Edward 1	63.0	38.0	40
King Edward 2	39.0	16.5	58
Redskin 1	7.8	4.2	46
Redskin 2	16.5	10.1	39

[1] *Verfahren und Sorte – Traitement et variété*
[2] *Rückstand im ungekochten Fleisch – Résidu dans la chair crue*
[3] *Rückstand im gekochten Fleisch – Résidu dans la chair cuite*
[4] *Rückstand, durch das Kochen entfernt – Résidu éliminé par la cuisson*
[5] *Gesottene Knollen – Cuisson par ébullition*
[6] *Chips – Cuisson en chips*

Tabelle 3. Einfluss des Kochens auf die Rückstände von 2-Aminobutan in begasten Kartoffeln.
Tableau 3. Effet de la cuisson sur les résidus de 2-aminobutane dans des pommes de terre soumises à la fumigation.

crisp makers. The potatoes were halved, one half being crisped before analysis and the other analysed to determine the level of 2-aminobutane present before crisping. The results shown in Table 3 indicate that a mean of just under 50% of the residue was removed. However, the percentage removed was substantially higher if the loss of water was taken into consideration.

Processing

Fumigated potatoes were commercially processed into potato flakes and granules. During this process the potatoes were cooked and dehydrated and only required the addition of boiling water four times the weight of the flakes or granules to prepare them for eating. Analysis showed that 83 mg/kg 2-aminobutane was present in the dried flake, whereas, in three samples of granules there were 72, 64 and 125 mg/kg present. These figures appear high but the levels on the reconstituted potato ready for eating would be 17 mg/kg for the flake, and 15, 13 and 25 mg/kg for the granule samples.

It is concluded that, at present, fumigation of ware potatoes cannot be carried out because very significant residues remain after peeling, boiling, crisping or processing into flakes or granules.

Residues of 2-aminobutane in crops grown from fumigated seed potatoes

There must be no translocation of chemicals from mother to daughter tubers when treated seed is grown for ware (table) potato production. In 1968, crops were harvested which had been grown from seed potatoes treated at 140 mg/kg three days after lifting in 1967. In 1969 crops were harvested from seed treated at levels of 200, 500 and 1000 mg/kg three days after lifting in 1968. In 1970, crops were harvested from seed treated three and fourteen days after lifting in 1969 at 200 mg/kg. Results of the analyses of all these crops and untreated material from the same source are given in Table 4.

The residues found in crops grown from treated seed are very small and are not greatly different from those found in untreated material, even when the mother tubers had been treated with 2-aminobutane at five times the recommended dosage. There is, therefore, no significant translocation of the chemical to daughter tubers, so that there is no hazard to consumers of crops grown from treated seed.

Results of disease control by fumigation

Over the years 1967–'71, 29 fumigation treatments were carried out on bulks of tubers mostly in the 5-tonne prototype chamber. The experiments were designed firstly to determine the phytotoxic dose; secondly to establish the dose to obtain good control; and thirdly to determine the efficiency of the treatment at different times after lifting.

Phytotoxicity

In the early experiments (Graham and Hamilton, 1970), it was found that a dosage of 200 mg/kg tubers had no phytotoxic effects other than slight browning of skinned

Table 4. Residues of 2-aminobutane in crops grown from fumigated seed tubers.

Year[1]	Treatment of seed tubers[2]	Number of cultivars used[3]	Number of stocks used[4]	Range of residue (mg/kg)[5]	Mean residue (mg/kg)[6]
1968	140 mg/kg	2	2	0.05	0.05
	Nil[7]	2	2	0.05	0.05
1969	200 mg/kg	3	4	0.04–0.12	0.08
	500 mg/kg	1	1	0.09–0.16	0.14
	1000 mg/kg	1	1	0.05–0.11	0.08
	Nil	3	4	0.03–0.11	0.07
1970	200 mg/kg (3 days)	2	2	0.01–0.10	0.05
	200 mg/kg (14 days)	2	2	0.01–0.06	0.03
	Nil	2	2	0.01–0.04	0.03

Limit of detection of 2-aminobutane 0.01 mg/kg – *Grenze der Nachweismöglichkeit von 2-Aminobutan 0,01 mg/kg – Limite de détection de 2 aminobutane 0,01 mg/kg*
Standard deviation 0.03 mg/kg – *Standardabweichung 0,03 mg/kg – Déviation standard 0,03 mg/kg*

[1] *Jahr – Année*
[2] *Behandlung von Pflanzknollen – Traitement des plantes*
[3] *Anzahl der verwendeten Sorten – Nombre de variétés utilisées*
[4] *Anzahl der verwendeten Partien – Nombre de stocks utilisés*
[5] *Rückstandsbereich – Niveau de résidu*
[6] *Mittlerer Rückstandswert – Quantité moyenne de résidu*
[7] *Keine – Nul*

Tabelle 4. Rückstände von 2-Aminobutan im Nachbau von begasten Pflanzknollen.
Tableau 4. Résidu de 2-aminobutane dans les récoltes issues de plants soumis à la fumigation.

areas. To determine the phytotoxic level, three separate lots of about 300 tubers of cv. *Majestic* were treated at dosages of 500, 1 000 and 5000 mg/kg. More marked browning of skinned areas was observed at the 500 mg/kg level, whereas at 1 000 mg/kg there was some lenticel pitting and very marked browning of damaged areas. At the 5000 mg/kg dosage chemical damage was severe and all eyes on the tubers were killed.

Immature and badly skinned tubers were, however, injured even at the 200 mg/kg dose, thus emphasising the need for treating only mature tubers.

Control of gangrene, skin spot and silver scurf by fumigation at different dosages at different times after lifting

Experiments with organomercury disinfectant solutions have shown that, in general, the longer the delay between harvesting and treatment, the poorer the degree of disease control. This probably results from changes in the skin, especially suberisation, making it increasingly impervious to the dipping solution.

By analogy, it seemed likely the same principle would apply to 2-aminobutane, so experiments were done at times varying from 1 to 30 days after lifting at dosages from

50 mg/kg to 200 mg/kg. In every case, fumigant was introduced in 30–40 min, and recirculation continued for a further 2 h. Tubers dug by elevator digger were received from various farms in bags. The farms were chosen on the basis that past experience had shown crops grown there were more likely to be affected by gangrene or skin spot than crops grown in other areas, which avoided having to use artificially inoculated material. In the early experiments tubers were not graded before treatment but later, tubers graded over a spool grader were used. After fumigation tuber samples taken from different positions in the chamber were stored in 0.5-tonne bulks under straw in a cool (5–8 °C) but frost-proof shed until February when the tubers were placed in trays to sprout in a warmer shed where the temperature varied between 9–15 °C. Assessments of rots were made on two occasions, once in February and again just before planting in April, whereas the skin diseases skin spot and silver scurf were assessed on washed samples of 50 or 100 tubers in April. Diseases were identified by symptoms but the diagnoses were checked by isolation from representative tuber samples. Some tests were also made using the eye plug method for skin spot, the eye plugs being incubated in moist chambers and examined microscopically for *Oospora pustulans* (Hide et al., 1968), which confirmed the presence or absence of viable skin spot fungus on treated material. For controls, 0.5-tonne bulks were stored and handled similarly, except that they were not loaded into and out of the chamber.

Results of the treatments on gangrene and skin spot are shown in Table 5; for simplicity the degree of skin spot infection is expressed only as the surface infection index (Boyd, 1957).

In one experiment the efficiency of 2-aminobutane at 200 mg/kg dosage was compared with dipping in a solution of methoxyethylmercuric chloride (MEMC) containing 100 mg of mercury per kg. Results are given in Table 6.

Control of silver scurf is shown by results given in Table 7.

Discussion

Our studies show that fumigation of potato tubers can be done easily in a simply made chamber fitted with a vaporiser and gas recirculation system.

The biological results illustrate the great efficiency of 2-aminobutane in controlling gangrene and skin spot at a dosage of 200 mg/kg. It is noteworthy that where gangrene did develop in fumigated material, it was often associated with severe mechanical damage, and such tubers would usually be removed at dressing in any case. Results of treatment at 50 mg/kg indicate that it is too low a dosage, and although only a few treatments were done at 100 mg/kg, there are indications that this dosage is sufficient to achieve satisfactory control. However, bearing in mind that, in commercial practice, the gas may not become equally distributed throughout the bulk, for instance as a result of the presence of soil, a dosage rate of 200 mg/kg is recommended. Like organomercury disinfectant solutions, best results for control of gangrene, skin spot and silver scurf were obtained if fumigation was done within three days of lifting, but even after 14 days the degree of control of both gangrene and skin spot was good at

Table 5. Results of experiments to test efficiency of treatment with 2-aminobutane at different dosages and different times of lifting on the incidence of gangrene and skin spot.

Date of lifting[1]	Cultivar[2]	Dosage mg/kg[3]	Number of days elapsed between harvest and treatment[4]	Number of tubers examined[5]	Percentage gangrene[6]	Skin spot surface infection index[7]
22.10.68	*Majestic 1*	200	1	1328	0.2	0.03
		nil[8]	–	1376	4.1	11.20
23.10.68	*King Edward 1*	200	1	1605	0.1	0
		nil	–	1458	5.8	9.4
13.11.68	*Redskin 1*	200	2	552	0.7	0
		nil	–	567	88.6	0
7.10.69	*Redskin 2*	200	1	1844	0.2	0
		200	14	2258	0.2	0.03
		nil	–	1957	5.0	5.60
15.10.69	*King Edward 2*	200	1	1869	0.4	0
		200	14	2202	0.7	0.03
		nil	–	2053	2.0	3.25
7.10.70	*King Edward 3*	50	2	2057	1.8	0.03
		100	2	1986	1.1	0
		200	2	2057	0.2	0
		50	13	2275	3.3	0
		200	13	2177	0.5	0.06
		50	27	2163	4.9	0.10
		200	27	2113	1.0	0.16
		nil	–	2657	8.1	9.15
13.10.70	*Redskin 3*	50	1	732	0.1	0
		100	1	708	0	0
		200	2	748	0	0
		50	14	727	0.1	0.03
		200	14	735	0	0
		50	30	743	0.1	0.16
		200	30	747	0.1	0.32
		nil	–	798	2.7	7.37
10.12.70	*Red Craigs Royal 1*	50	5	880	36.8	0.19
		200	5	874	13.8	0.09
		nil	–	334	79.6	3.63
10.12.70	*Majestic 2*	50	3	1104	1.4	0.48
		200	3	1137	0.2	0.25
		nil	–	443	7.7	4.09
1.10.71	*King Edward 4*	50	1	2153	0	0.06
		200	1	2123	0	0
		50	29	2328	0	0.19
		200	29	2383	0	0.25
		nil	–	1867	0.2	3.84

Table 6. Effectiveness of 2-aminobutane fumigation compared with methoxyethylmercuric chloride (MEMC) disinfectant solution in controlling gangrene.

Cultivar[1]	Treatment[2]	Number of tubers examined[3]	Percentage gangrene[4]
Majestic	2-aminobutane	227	4.8
	MEMC	754	6.1
	nil[5]	195	33.3
Redskin	2-aminobutane	291	7.2
	MEMC	314	15.0
	nil	329	43.4

[1] *Sorte – Variété*
[2] *Behandlung – Traitement*
[3] *Anzahl untersuchter Knollen – Nombre de tubercules examinés*
[4] *Prozent Phoma-Knollenfäule – Pourcentage de gangrène*
[5] *Null – Nul*

Tabelle 6. Wirksamkeit von 2-Aminobutan-Begasung, verglichen mit der Desinfektionsmittellösung Quecksilbermethoxyäthylchlorid für die Bekämpfung von Phoma-Knollenfäule.
Tableau 6. Efficacité de 2-aminobutane en fumigation comparée au chlorure de méthoxyéthylmercurique en solution dans la butte contre la gangrène.

the 200 mg/kg dosage rate. After about one month the treatment was less efficient, though, even so, control of gangrene and skin spot was still reasonably good. Control of silver scurf was always poorer than with gangrene and skin spot and results of delaying treatment after harvest are especially well illustrated by this disease (Table 7). In commercial practice, fumigation within 14 days of harvest is recommended, allowing more time for lifting, grading and handling, than in the case of organomercury dips where treatment is recommended to be done within 3 days of lifting. The reason why 2-aminobutane remains effective for a longer time is probably, at least in part, a

◀
[1] *Erntedatum – Date d'arrachage*
[2] *Sorte – Variété*
[3] *Dosis – Dose*
[4] *Anzahl verflossener Tage zwischen Ernte und Behandlung – Nombre de jours entre la récolte et le traitement*
[5] *Anzahl untersuchter Knollen – Nombre de tubercules examinés*
[6] *Prozent Phoma-Knollenfäule – Pourcentage de gangrène*
[7] *Index für Befall mit Tüpfelfleckigkeit (Oberfläche) – Index de surface infectée d'oosporiose*
[8] *Null – Nul*

Tabelle 5. Ergebnisse von Versuchen, die Wirksamkeit von Behandlungen mit 2-Aminobutan bei verschiedener Dosierung und unterschiedlichen Erntezeiten auf den Befall mit Phoma-Knollenfäule und Tüpfelfleckigkeit zu testen.
Tableau 5. Résultats de tests d'efficacité de traitements au 2-aminobutane à différentes doses et différentes époques après l'arrachage sur les manifestations de gangrène et d'oosporiose.

Table 7. Control of silver scurf by fumigation with 2-aminobutane.

Cultivar[1]	Treatment[2]	Percentage skin cover with silver scurf[3]
King Edward	200 mg/kg 2 days after lifting[4]	13.1
	200 mg/kg 29 days after lifting	41.6
	nil[5]	71.6
Pentland Crown	200 mg/kg 2 days after lifting	18.4
	200 mg/kg 29 days after lifting	31.8
	nil	47.1

[1] *Sorte – Variété*
[2] *Verfahren – Traitement*
[3] *% mit Silberschorf bedeckte Schale – Pourcentage de peau couverte de gale argentée*
[4] *Tage nach der Ernte – Jours après l'arrachage*
[5] *Null – Nul*

Tabelle 7. Bekämpfung von Silberschorf durch Begasung mit 2-Aminobutan.
Tableau 7. Le traitement de la gale argentée par fumigation avec le 2-aminobutane.

reflection of penetration of the skin in which the fungi occur. In mature, fully suberised tubers of the cv. *Majestic* more than 90% of methoxyethylmercuric chloride is contained within the first millimetre of peel (Hamilton and Ruthven, 1967) whereas only 60–70% of 2-aminobutane is contained within the first millimetre.

The experiment comparing the efficacy of 2-aminobutane with a methoxyethylmercuric chloride dip shows that 2-aminobutane is the more effective (Table 6). Even so the results with 2-aminobutane were poorer than expected, probably due to the fact that treatments were done in a small chamber where distribution of the gas was uneven.

Experience has shown that control of gangrene by organomercurial dipping solutions can prove more difficult with tubers lifted very late in the season. The reason for this is not clear, but it may be due to poorer penetration because the skin has become more impervious or because the infection has become more deep-seated by then, or perhaps for both reasons. To see if the same problem would arise with 2-aminobutane, a stock of cv. *Red Craigs Royal* (*Red Craigs Royal* 1 in Table 5) and a stock of cv. *Majestic* (*Majestic* 2 in Table 5) were obtained from an area in N.E. Scotland in December. The *Red Craigs Royal* untreated tubers developed nearly 80% gangrene, while fumigation five days after lifting at 200 mg/kg reduced the loss to around 14%. This is not as good a control as in other experiments, but, even so, fumigation would prove to be economically well worth while in such cases.

The effect of treatments on sprouting, growth and yield of tubers will be described elsewhere, as field trials are still in progress. However, experience over four years with several cultivars shows that, in general, 2-aminobutane treatment causes more sprouts to develop on tubers, thus there are more stems per plant and a greater proportion of

smaller (mainly seed size tubers) are produced as compared with healthy untreated tubers. Yields obtained from treated tubers generally have either been the same as, or greater than, those from untreated tubers. These effects are comparable with results obtained with organo-mercury disinfectant solutions (Boyd and Penna, 1967).

Treatment with 2-aminobutane did not control tuber blight (caused by *Phytophthora infestans*), dry rot (*Fusarium solani* var. *caeruleum*), nor did it kill the sclerotia of *Rhizoctonia solani*. Its effect on other tuber diseases is not yet known.

Experiments are continuing, present work concentrating on the efficiency of fumigation when tubers are treated after grading in February. Investigations are also being made on the degree to which the crops grown from treated seed are infected with gangrene, skin spot and silver scurf fungi.

The use of 2-aminobutane for fumigation of seed potatoes has been patented in the United Kingdom (patent specification 1268490) and in Eire. Fumigation has been cleared for safety under the British Pesticides Safety Precautions Scheme for use on seed potatoes. It must not be used on ware (table) potatoes.

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Zusammenfassung

Anwendung von 2-Aminobutan als Räuchermittel zur Bekämpfung von Phoma-Knollenfäule, Tüpfelfleckigkeit und Silberschorf von Kartoffelknollen

Jedes Jahr können Pilzkrankheiten an Kartoffelknollen beträchtliche Verluste verursachen. In Schottland ist die Phoma-Knollenfäule, verursacht durch den Pilz *Phoma exigua* var. *foveata*, die bedeutendste Krankheit, doch ist die Tüpfelfleckenkrankheit, hervorgerufen durch *Oospora pustulans*, ebenfalls wichtig, vor allem weil sie die Keime befällt. Silberschorf, verursacht durch *Helminthosporium solani*, ist sehr verbreitet, aber obwohl er die Keime nicht befällt, werden durch ihn die Knollen missgebildet, und er verursacht Welke infolge des Wasserverlustes durch die beschädigte Schale.

Begasung von Knollen mit 2-Aminobutan (sek-Butylamin)-Dampf in einer Dosierung von 200 mg/kg innerhalb 14 Tage nach der Ernte wurde als ein sehr gutes Mittel zur Bekämpfung von Phoma-Knollenfäule und Tüpfelfleckigkeit (Tabelle 5) befunden. In zwei Versuchen ergab die Begasung eine bessere Bekämpfung von Phoma-Knollenfäule als das Eintauchen in eine Lösung von Quecksilbermethoxyäthylchlorid, das 100 mg/kg Quecksilber enthält (Tabelle 6). Eine gewisse Bekämpfung der Silberschorfkrankheit wurde mit 2-Aminobutan erzielt (Tabelle 7), aber die Ergebnisse waren immer schlechter als bei Phoma-Knollenfäule und Tüpfelfleckigkeit. Phytophthora-Knollenfäule (*Phytophthora infestans*) und Fusarium-Trockenfäule (*Fusarium solani* var. *caeruleum*) wurden durch die Begasung nicht bekämpft, und auch auf die Sklerotien von *Rhizoctonia solani* hatte die Begasung keinen Einfluss. Die Beurteilung der Fäulen wurde an zwei Terminen vorgenommen, einmal im Februar und dann wieder im April, während die Schalenkrankheiten, Tüpfelfleckigkeit und Phoma-Knollenfäule im April an gewaschenen Mustern von 50 oder 100 Knollen beurteilt wurden. Der Bau der Gaskammern (dargestellt in Abb. 1 und 2) wird kurz beschrieben und der Vorgang der Begasung diskutiert. Die Kammern sind für Gas-Innenkreislauf eingerichtet, das

System ist verbunden mit einem Gebläse und einem Verdampfer. Da das 2-Aminobutan-Luftgemisch durch einen losen Kartoffelhaufen dringt, wird die Chemikalie von den unteren Knollenschichten aufgenommen; aber da der Innenkreislauf anhält, findet eine Abgabe und Wiederaufnahme statt, so dass der Wirkstoff, wie sich bei Rückstandsanalysen gezeigt hat, möglicherweise gleichmässig im Haufen verteilt wird. Es wurde festgestellt, dass bei einer Dosis von 200 mg/kg in 30–40 Minuten und bei Luftumwälzung während weiteren zwei Stunden die Rückstände in Knollenmustern von unten und oben im Haufen ähnlich waren (Tabelle 1).

Rückstände von 2-Aminobutan waren in signifikantem Ausmass vorhanden in geschälten (Tabelle 2) und gesottenen Kartoffeln sowie in Chips (Tabelle 3), ferner in Flocken und Granulaten, die aus begasten Kartoffeln hergestellt wurden, so dass das Verfahren nur bei Pflanzkartoffeln angewendet werden kann. Analysen des Nachbaus von behandelten Knollen zeigte, dass sie keine signifikanten Rückstände enthalten (Tabelle 4).

2-Aminobutan wird leicht verdampft (Siedepunkt 63 °C) und ist schwach giftig; die Giftigkeit beruht in erster Linie auf seiner Alkalinität. Gewisse Vorsichtsmassnahmen müssen getroffen werden, wenn diese Substanz zur Begasung von Kartoffeln verwendet wird; sie sind in einer formellen Gebrauchsanweisung beschrieben, die von den Autoren bezogen werden kann.

Résumé

L'utilisation du 2-aminobutane en fumigation par le traitement des maladies de la gangrène, de l'oosporiose et de la gale argentée des tubercules de pomme de terre

Les maladies fongiques des tubercules de pomme de terre peuvent occasionner des pertes annuelles considérables. En Ecosse, la gangrène, causée par le champignon *Phoma exigua* var. *foveata*, est la maladie la plus grave mais, l'oosporiose, causée par *Oospora pustulans*, est également importante, spécialement quand elle attaque les germes. La gale argentée, causée par *Helminthosporium solani*, est très commune, mais bien que n'attaquant pas les germes, elle détériore l'aspect des tubercules et provoque leur ramolissement par suite de la perte d'eau au travers de la peau endommagée.

On a trouvé que la fumigation des tubercules avec la vapeur de 2-aminobutane (sec-butylamine), à la dose de 200 mg/kg dans les 14 jours qui suivent l'arrachage avait une très bonne efficacité dans la lutte contre la gangrène et l'oosporiose (tableau 5); de plus, dans deux essais, le résultat du traitement contre la gangrène a été supérieur au trempage dans une solution du chlorure de méthoxyethylmercurique à 100 mg/kg de mercure (tableau 6). Le 2-aminobutane a donné certains résultats dans le traitement de la gale argentée (tableau 7), mais ceux-ci sont toujours inférieurs à ceux obtenus contre la gangrène et l'oosporiose. La fumigation n'a aucun effet contre le mildiou du tubercule (*Phytophthora infestans*) et la pourriture sèche (*Fusarium solani* var. *caeruleum*), pas plus que contre les sclérotes du *Rhizoctonia solani*. La détermination des pourritures a été faite deux fois, une première fois en février et une seconde en avril, tandis que les déterminations des maladies de la peau, oosporiose et gangrène, ont été effectuées en avril sur des échantillons lavés de 50 ou 100 tubercules.

Les auteurs décrivent brièvement la construction des chambres de fumigation (voir fig. 1 et 2) et expliquent le mécanisme de fumigation. Les chambres sont équipées pour la circulation forcée du gaz, grâce à un ventilateur et un vaporisateur. Au moment où le mélange air – 2-aminobutane traverse le tas de pommes de terre, la substance chimique est absorbée par les couches inférieures de tubercules, mais grâce à la circulation forcée, il se produit des phénomènes de 'désaborption' et de 'réabsorption' de telle sorte que, finalement, la substance chimique se répartit d'une manière égale dans la masse de tubercules, comme l'indique l'analyse des résidus. Il a été démontré que si on applique la dose de 200 mg/kg pendant 30–40 minutes et qu'on réalise la circulation forcée pendant un minimum de 2 heures, les résidus dans les échantillons de tubercules sont identiques de la base au sommet du tas (tableau 1).

Les résidus de 2-aminobutane sont présents en quantités significatives dans les tubercules

pelés (tableau 2), bouillis et transformés en chips (tableau 3), de même dans les flocons et granulés fabriqués à partir de pommes de terre traitées, de sorte que le traitement ne peut s'appliquer qu'aux plants. L'analyse des récoltes issues de plants traités ne révèle aucune quantité significative de résidu (tableau 4).

Le 2-aminobutane se vaporise aisément (63 °C), est modérément toxique, la toxicité étant due en premier lieu à l'alcalinité. Il y a lieu de prendre certaines précautions dans l'emploi de cette substance dans la fumigation des pommes de terre, qui sont décrites dans le code d'emploi officiel que les auteurs peuvent fournir.

References

Anonymous, 1960. Bulk storage of potatoes in buildings. *Bull. Min. Agric. Fish. Fd* 173. HMSO.

Anonymous, 1966. Technical report on Tutane. Elanco Products Co, Indianapolis, Indiana, USA.

Boyd, A. E. W., 1957. Field experiments on potato skin spot disease caused by *Oospora pustulans* Owen and Wakef. *Ann. appl. Biol.* 45: 284–292.

Boyd, A. E. W. & R. J. Penna, 1967. Seed tuber disinfection. *Proc. 4th Br. Insectic. Fungic. Conf.* 1: 294–302.

Day, E. W., F. J. Holzer, J. B. Tepe, J. W. Eckert & M. J. Kolbezen, 1968. Determination of sec-butylamine residues in fruit. *J. Ass. off. analyt. Chem.* 51: 39–44.

Eckert, J. W., M. L. Rahm & M. J. Kolbezen, 1970. Fungistatic activity of non-aromatic amines. *Phytopathology* 60: 1533–1534.

Graham, D. C. & G. A. Hamilton, 1970. Control of potato gangrene and skin spot diseases by fumigation of tubers with sec-butylamine. *Nature, Lond.* 227: 297–298.

Hamilton, G. A. & A. D. Ruthven, 1967. Residual mercury content of seed potatoes treated with organo-mercury disinfectant solutions. *J. Sci. Fd Agric.* 18: 558–563.

Hide, G. A., J. M. Hirst & R. L. Griffith, 1969. Control of potato tuber diseases with systemic fungicides. *Proc. 5th Br. Insectic. Fungic. Conf.* 2: 310–314.

Hide, G. A., J. M. Hirst & G. A. Salt, 1968. Methods of measuring the prevalence of pathogenic fungi on potato tubers. *Ann. appl. Biol.* 62: 309–318.

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Fumigation of bulk-stored potatoes with 2-aminobutane for control of gangrene, skin spot and silver scurf diseases

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Summary

In October, 1970, a 35-tonne bulk of potato tubers cv. *Pentland Crown*, containing samples of cvs *King Edward* and *Redskin*, was fumigated with 2-aminobutane in a bin store fitted for internal recirculation of air. After fumigation, some of the treated samples were transferred to other 35-tonne lots along with untreated samples, for subsequent storage in different environments.

Analysis of 2-aminobutane residues in tubers showed that even distribution had been achieved throughout the treated bulk. Assessment of diseases after about five months' storage showed that fumigation gave excellent control of gangrene and skin spot, and partial control of silver scurf under a wide range of environments. Compared with the marked effects of fumigation, the influence of the different storage environments on levels of gangrene and skin spot in untreated tubers was slight.

Introduction

Investigations into the chemical control of potato tuber diseases at the Agricultural Scientific Services have shown that the diseases gangrene (*Phoma exigua* var. *foveata*) and skin spot *(Oospora pustulans)* were well controlled by fumigation with gaseous 2-aminobutane (sec-butylamine) in a five tonne chamber fitted with a forced-draught air recirculation system incorporating a vaporizer. In addition, some control of silver scurf *(Helminthosporium solani)* was obtained (Graham and Hamilton, 1970; Graham et al., 1973). Other experiments in a vertical cylindrical chamber showed that 2-aminobutane could be moved from the bottom to the top of a column of tubers 3 m high.

As commercial potato stores of large capacity fitted with internal recirculation systems seemed adaptable for fumigation, an opportunity was taken in 1970 to see if fumigation could be done successfully in the experimental stores of the Edinburgh School of Agriculture. These comprise a specialized building containing four 35-tonne bins, each possessing a fan and ventilation system to provide either through ventilation or internal circulation; together with a 35-tonne portion of an adjacent standard store possessing an uninsulated roof, brick walls, gable openings, below-ground duct and mobile fan. The potatoes in the standard store are covered with straw. Both stores are fitted with a frost-guard thermostat, but the specialized store

also contains differential thermostats and refrigeration and humidification equipment (Nash and Lennard, 1970, 1973). To test the efficiency of the fumigant for disease control treated material was subsequently kept under the wide range of environmental conditions obtainable in the stores, from simple storage without forced air ventilation to storage involving continuous recirculation with artificially cooled, humidified air.

Materials and methods

Harvesting, sampling, filling and storage procedures were similar to those used previously in experiments not involving fumigation (Nash and Lennard, 1970, 1973). A stock of *Pentland Crown*, grown nearby on a School of Agriculture farm, was used to fill all stores. Lifting and filling began on 14 October and was completed on 20 October; unloading was done 5 months later. Storage treatments are summarized in Table 1.

When the bins were full, the height of the bulk of tubers was about 4 m. The amounts of soil in the bulks of *Pentland Crown* at the end of storage were not determined, but because of dry lifting conditions there was very little soil adhering to the tubers.

Table 1. Storage treatments.

Storage[1]	Storage treatment[2]*	Ventilation[3] (h)	Storage temperature[4] (°C)
Specialized store[5]			
Bin[6] 1	OAC	222	3–5
Bin 2	RAA	3178	3–5
Bin 3	RAA + H	3248 + 3300 H	3–5
Bin 4	F + OAC	243	3–5
Standard store[7]			
Lot[8] 5	UV	39**	7–10

*F = Fumigation – *Begasung – Fumigation*; OAC = Outside air cooling – *Aussenluftkühlung – Refroidissement par l'air extérieur;* RAA = Recirculation of artificially cooled air – *Interne Umwälzung künstlich gekühlter Luft – Recirculation de l'air refroidi artificiellement;* H = Humidification – *Luftbefeuchtigung – Humidification;* UV = Unventilated – *Nicht belüftet – Non ventilé*

**39 hours of ventilation given at the beginning of the storage season; no ventilation thereafter – *39 Stunden belüftet zu Beginn der Lagerperiode, nachher keine Belüftung mehr – 39 heures de ventilation au début de la saison de conservation; aucune ventilation ultérieure*

[1] *Lager – Conservation*
[2] *Lagerungsverfahren – Traitements de conservation*
[3] *Belüftung – Ventilation*
[4] *Lagertemperatur – Température de conservation*
[5] *Speziallager – Magasin spécial*
[6] *Boxe – Box*
[7] *Standardlager – Magasin standard*
[8] *Haufen – Lot*

Tabelle 1. Lagerungsverfahren.
Tableau 1. Traitements de conservation.

Samples for disease assessment and residue analyses

During the filling operation a large number of samples of tubers was buried in the bulk for disease assessment (and weight loss) after storage. Each sample comprised 40 tubers in a net bag and each storage treatment contained 30 bags, made up of 18 bags of cv. *Pentland Crown*, six of cv. *Redskin* and six of cv. *King Edward*. The *Redskin* and *King Edward* tubers were taken from farms known to produce material likely to be affected by gangrene and skin spot, to avoid using artificially inoculated tubers. After fumigation in bin 4, six bags of *Pentland Crown*, six of *King Edward* and six of *Redskin* were transferred from it to each of bins 2 and 3 and the standard store, 5. These bags had been placed on top of the bulk in bin 4 before fumigation. None needed to be placed in the control bin 1 because the ventilation management was the same as that for bin 4. Data on weight loss in storage will be discussed elsewhere.

In addition to the material for disease and weight loss assessment, samples of *Pentland Crown* for 2-aminobutane residue analysis were placed in the duct below bin 4 and in the lower, middle and top layers of the bulk, making 30 samples in all. Bags in the top layer were removed at intervals during fumigation and others at the end of fumigation together with the samples in the duct. The samples within the bulk remained until the bin was emptied.

Method of fumigation

Experiments in the 5-tonne fumigation chamber showed that an air flow of about 5.6 m^3 min^{-1} $tonne^{-1}$ moved fumigant absorbed by the lower layers of tubers up through the bulk to the top by the desorption and resorption process described by Graham et al. (1973). Because the 37.5 cm diameter fans already installed in the bins could provide an air flow of only about 1.7 m^3 min^{-1} $tonne^{-1}$, the fan in bin 4 was replaced by a 47.5 cm diameter fan and the pitch of the fan blades increased from 8° to 31°. The 47.5 cm fan produced an air flow of about 135 m^3 min^{-1} when the bin was empty, equivalent to about 3.9 m^3 min^{-1} $tonne^{-1}$ in the full bin, assuming no reduction in air flow due to restriction caused by the tubers. Measurements when the bin was full showed that the airflow was reduced to about 2.8 m^3 min^{-1} $tonne^{-1}$, but it was decided to go ahead with the fumigation with this rate of flow as very substantial alterations to fan and motor would have been needed to increase it. After fumigation, the 37.5 cm diameter fan was put back to provide normal ventilation.

A water-jacketed vaporizer (described by Graham et al., 1973) was fitted into the duct beneath the bin. The doors of the bin were sealed with adhesive tape before fumigation. The fumigant was pumped into the vaporizer at 220 ml/min with a peristaltic pump; 10.6 l of 2-aminobutane was required to give the recommended dose of 200 mg/kg (Graham et al., 1973) and took 48 min to apply. Circulation of air and fumigant was continued for 2 h after all the fumigant had been introduced.

Gas sampling and residue analysis

Before filling bin 4, small-bore polythene gas sampling pipes were put into the duct below the bin and, during filling, more pipes were inserted into the bulk and led

outside so that both the vertical and horizontal distribution of gaseous 2-aminobutane could be followed in the air spaces between potatoes during and after application of gas. The 2-aminobutane concentration was measured as Graham et al. (1973) described, with ammonia detector tubes. The gas chromatographic method used for residue analysis was also the same as that described by Graham et al. (1973).

Results

Measurements of free fumigant

Measurements of free fumigant made by drawing samples through the polythene tubes inserted into bin 4 showed that the gas moved from bottom to top of the bulk quite rapidly. This suggested good distribution of the fumigant and that a 2-hour recirculation period would be sufficient to achieve a satisfactory result.

Residues of 2-aminobutane

The degree to which equal distribution of 2-aminobutane took place throughout the bulk of tubers was determined by residue analysis. Results are given in Table 2.

Table 2. Residues of 2-aminobutane in *Pentland Crown* tubers

Sample No[1]	Position in bin 4[2]	Time of sampling[3]	Range of residues[4] (mg/kg)	Mean residue (mg/kg)
1–2	top layer[6]	after 50% of dose applied[10]	2.3–6.8	4.6
3–4	top layer	after 100% dose applied[10]	57–114	86
5–6	top layer	after 1 h further recirculation[11]	103–112	108
7–8	duct below bin[7]	at end of fumigation[12]	156–236	196
9–17	top layer	at end of fumigation[1]	124–134	161
18–21	top layer	at unloading of bin[13]	95–122	111
22–23	middle layer[8]	at unloading of bin	64–113	88
26–30	bottom layer[9]	at unloading of bin	58–130	100

[1] *Muster Nr. – No d'échantillage*
[2] *Stelle in Boxe 4 – Position dans le box 4*
[3] *Zeitpunkt der Musterentnahme – Moment de l'échantillonnage*
[4] *Bereich der Rückstände – Classement des résidus*
[5] *Rückstand, Mittelwert – Moyenne de résidus*
[6] *Oberste Schicht – Couche supérieure*
[7] *Luftkanal unter dem Boxe – Conduit en-dessous du box*
[8] *Mittlere Schicht – Couche du milieu*
[9] *Unterste Schicht – Couche inférieure*
[10] *Nach Anwendung von 50% (100%) der Dosis – Après application de 50% (100%) de la dose*
[11] *Nach einer Stunde weiterer interner Luftumwälzung – Après 1 heure de recirculation*
[12] *Am Ende der Begasung – A la fin de la fumigation*
[13] *Beim Entleeren der Boxe – Au déchargement des box*

Tabelle 2. Rückstände von 2-Aminobutan in Knollen der Sorte *Pentland Crown*.
Tableau 2. Résidus de 2-aminobutane dans les tubercules *Pentland Crown*.

The residue levels in samples taken during and at the end of fumigation (Nos 1–17), showed there was good horizontal distribution at the top of the bin. Vertical distribution also seemed to have been satisfactory because samples taken from various positions in the top of the bin had a mean residue level of 161 mg/kg whereas a mean level of 196 mg/kg was found on samples taken from the duct below the bin.

The samples buried in the middle and bottom positions of the bulk were analysed 5 months after fumigation when the bin was emptied and further samples taken from the top of the bin were analysed for comparison (Nos 18–30). Some reduction in the residue levels had occurred, but the mean levels of 111, 88 and 100 mg/kg in the top, middle and bottom samples, respectively, confirmed that gas distribution had been satisfactory throughout the bulk.

Disease assessments

Preliminary observations were made on the incidence of disease at the time of loading the stores, using samples of 150 tubers of each cultivar. Only slight amounts of surface diseases were visible, and in no case was there more than 2% of tubers affected by blight (*Phytophthora infestans*) or bacterial soft rot (*Erwinia carotovora* var. *atroseptica*).

Assessment of diseases affecting tubers of each cultivar at the end of the 5-month storage period were based on an examination of two 40 tuber samples from each of the top, middle and bottom layers of the bins and the standard store. Additionally, six 40-tuber samples of each cultivar, which had been transferred from bin 4 after fumigation and stored in the top layers of tubers in bins 2 and 3 and the standard store 5, were examined to see if the different environments had any effect on disease development. The main diseases that developed in storage were gangrene, skin spot and silver scurf.

The gangrene results are summarized in Table 3. There was a high incidence of gangrene in the untreated tubers of *King Edward* in all storage environments, but less in *Pentland Crown* and *Redskin*. In the fumigated bin, there was no gangrene in any of the samples and there was only an occasional occurrence of this disease in some fumigated samples transferred to other storage environments.

Table 4 shows that whereas skin spot was present in slight to moderate amounts in untreated potatoes of all cultivars with no marked differences between different storage environments, the disease was absent from material in the fumigated bin and only very occasionally present in samples transferred to other storage environments. For simplicity, the data are presented only as surface and eye infection indices (Nagdy and Boyd, 1965). The results of *O. pustulans* assessments on samples of eye plugs and stolon scars, by the method of Hide et al. (1968), confirmed the high level of control of this fungus by 2-aminobutane; samples from the fumigated bin were completely free from *O. pustulans*.

Table 5 shows that there was some control of silver scurf but that this disease developed most on both treated and untreated tubers in the standard store, where temperatures were higher (Table 1).

Table 3. Percentage of tubers affected by gangrene in fumigated and unfumigated material in relation to storage treatment.

Storage treatment[1]	*Pentland Crown*		*Redskin*		*King Edward*	
	fumigated[2]	untreated[3]	fumigated	untreated	fumigated	untreated
Outside air cooling[4]	0.0	0.0	0.0	1.7	0.0	50.2
Artificial cooling[5]	0.0	1.3	0.0	2.9	0.4	44.2
Artificial cooling and humidification[6]	0.0	6.8	0.0	0.0	3.3	36.0
Unventilated[7]	0.4	1.7	0.0	0.0	0.0	37.5

[1] *Lagerungsverfahren – Traitement de stockage*
[2] *Begast – Fumigé*
[3] *Unbehandelt – Non traité*
[4] *Aussenluftkühlung – Refroidissement à l'air extérieur*
[5] *Künstliche Kühlung – Refroidissement artificiel*
[6] *Künstliche Kühlung und Luftbefeuchtigung – Refroidissement artificiel et humidification*
[7] *Nicht belüftet – Non ventilé*

Tabelle 3. Durch Phoma-Knollenfäule befallene Knollen (in %) in begasten und unbegasten Material im Verhältnis zum Lagerungsverfahren.
Tableau 3. Pourcentage de tubercules atteints de gangrène dans du matériel fumigé et non fumigé en fonction du traitement de stockage.

Table 4. Surface infection and eye infection indices for skin spot in relation to fumigation and storage treatments. (Figures in brackets refer to eye infection indices)

Storage treatment[1]	*Pentland Crown*		*Redskin*		*King Edward*	
	fumigated[2]	untreated[3]	fumigated	untreated	fumigated	untreated
Outside air cooling[4]	0.0 (0)	7.3 (34)	0.0 (0)	13.3 (54)	0.0 (0)	8.6 (63)
Artificial cooling[5]	0.0 (0)	7.6 (42)	0.0 (0)	11.3 (54)	0.0 (0)	8.7 (62)
Artificial cooling and humidification[6]	0.0 (0)	5.9 (32)	<0.1 (<1)	8.7 (40)	<0.1 (<1)	7.9 (51)
Unventilated[7]	0.0 (0)	7.1 (41)	0.0 (0)	7.4 (47)	0.4 (4.9)	8.5 (61)

[1–7] *Siehe Tabelle 3 – Voir tableau 3*

Tabelle 4. Index der Oberflächeninfektion und der Augeninfektion mit Tüpfelfleckigkeit in Beziehung zur Begasung und zu den Lagerungsverfahren. (Zahlen in Klammern beziehen sich auf den Index für die Augeninfektion.)
Tableau 4. Cotes des surfaces et des yeux infectés d'oosporiose en fonction de la fumigation et des traitements de stockage. (Les chiffres entre parenthèses de rapportent à l'infection des yeux.)

Table 5. Mean percentage surface area affected by silver scurf in relation to fumigation and storage treatments.

Storage treatment[1]	*Pentland Crown*		*Redskin*		*King Edward*	
	fumigated[2]	untreated[3]	fumigated	untreated	fumigated	untreated
Outside air cooling[4]	1	11	9	44	9	64
Artificial cooling[5]	1	3	8	18	8	17
Artificial cooling and humidification[6]	1	4	6	28	13	31
Unventilated[7]	6	52	28	69	60	90

[1–7] *Siehe Tabelle 3 – Voir tableau 3*

Tabelle 5. Mittlerer Prozentsatz der von Silberschorf befallenen Oberfläche in Beziehung zur Begasung und zu den Lagerungsverfahren.

Tableau 5. Pourcentage moyenne de la surface atteinte de gale argentée en fonction de la fumigation et des traitements de stockage.

Microscopical assessments by the eye plug method showed the same general effects of storage treatment and fumigation as did visual assessments, the control of the disease by cool storage or fumigation being most effective in *Pentland Crown* which showed the lowest levels of infection at the time of filling the stores.

Although the fumigated tubers were cleaner than untreated tubers because of the control of silver scurf, the appearance of *King Edward* and *Redskin* was somewhat marred by the presence of small necrotic areas around the lenticels. In both cases the material had been received in plastic bags several days before putting in store and the lenticels had proliferated in the moisture-saturated air. There was also some browning of mechanically damaged areas which had not healed before treatment.

Discussion

The fumigation process

Residue analyses show that application of the fumigant over about 50 min followed by a further 2-hour circulation at an air flow of about 2.8 m^3 min^{-1} $tonne^{-1}$ was enough to give a satisfactory distribution. After one hour of recirculation, the residue levels in tubers at the top of the bin were just over 100 mg/kg, but this increased to about 160 mg/kg after another hour. Analyses of tubers removed from the bin at the end of storage confirmed that satisfactory distribution had been achieved throughout the bulk. Surprisingly, the build-up of fumigant at the top of the bin during application was much quicker than in earlier tests with the 5-tonne chamber, even although the velocity of the air up through the tubers (about 7.7 m/min) was the same in the bin as in the chamber. (The velocity in the 5-tonne chamber resulted in an air flow of 5.6 m^3 min^{-1} $tonne^{-1}$ whereas the same velocity in the bin resulted in an air flow of 2.8 m^3 min^{-1} $tonne^{-1}$ because of the different sizes and shapes of the chamber and

bin). More work needs to be done to discover how to effect rapid and even distribution of 2-aminobutane in stores other than the kind used in this investigation, especially those which allow no recirculation. So far, the best ways of achieving good gas distribution in different stores and chambers can only be found empirically, which may require a time-consuming series of tests.

Disease control

The results for gangrene and skin spot again demonstrate how well both diseases can be controlled by 2-aminobutane fumigation. Compared with the marked effects of fumigation, the influence of the different storage treatments on the incidence of gangrene and skin spot in both treated and untreated tubers was slight. All samples from the fumigated bin were free from the two diseases whereas their presence in fumigated samples transferred to other environments and exposed to possible contamination from untreated tubers was negligible.

The incidence of silver scurf was also reduced, but the treatment was less effective than for gangrene and skin spot. This applied especially where the storage temperature was high, and in stocks where the prevalence of the fungus on tubers was high at the time of filling the stores.

Although the fumigant had an injurious effect on proliferated lenticels there was no evidence that the keeping quality of treated tubers was impaired. However, to preserve the good appearance of potatoes, it is recommended that the treatment of immature or freshly damaged tubers should be avoided, otherwise discolouration can occur.

It is concluded that 2-aminobutane can be used to fumigate large bulks of seed tubers to obtain very good control of gangrene and skin spot and some control of silver scurf, in a building designed for closed air circulation.

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Zusammenfassung

Begasung von lose gelagerten Kartoffeln mit 2-Aminobutan zur Bekämpfung von Phoma-Knollenfäule, Tüpfelfleckigkeit und Silberschorf

Im Oktober 1970 wurde 2-Aminobutan-Gas mittels Luftumwälzung in ein 35 Tonnen fassendes Lager mit Knollen der Sorte *Pentland Crown* eingeführt; das Lager enthielt auch Muster der Sorten *King Edward* und *Redskin*. Nach der Begasung wurden einige der behandelten Muster in andere 35-Tonnen-Lager verbracht,wo verschiedene Lagerungsverfahren angewendet wurden (Tabelle 1).

Das 2-Aminobutan wurde in einer Dosis von

200 mg/kg bei Beachtung einer Luftumwälzung von ungefähr 2,8 m^3 min^{-1} $Tonne^{-1}$ während ca. 50 Minuten angewendet. Darauf wurde während weiteren 2 Stunden die Luft im Lager umgewälzt. Messungen der Gaskonzentrationen zwischen den Knollen während der Begasung zeigten, dass das 2-Aminobutan wahrscheinlich gleichmässig durch den Haufen verteilt war. Dies wurde durch Rückstandsanalysen bestätigt (Tabelle 2).

Die Ergebnisse der Beurteilungen auf Befall mit Krankheiten nach fünfmonatiger Lagerung zeigten, dass 2-Aminobutan ein gutes Bekämpfungsmittel gegen Phoma-Knollenfäule (Tabelle 3) und Tüpfelfleckigkeit (Tabelle 4) war. Die Muster im begasten Haufen waren vollständig frei von diesen Krankheiten, und ihr Vorkommen in den begasten Mustern, die zu andern Haufen bzw. Lagerungsverfahren gebracht wurden, war unbedeutend. Verglichen mit den ausgeprägten Wirkungen der Begasung war der Einfluss der verschiedenen Lagerungsverfahren auf das Ausmass von Phoma-Knollenfäule und Tüpfelfleckigkeit bei den nicht begasten Knollenmustern gering. Die Begasung reduzierte das Ausmass der Infektion mit Silberschorf (Tabelle 5), aber der Grad der Bekämpfung war kleiner als bei Phoma-Knollenfäule und Tüpfelfleckigkeit, besonders bei hoher Lagertemperatur und bei Kartoffeln, bei denen zur Zeit der Einlagerung des Pilz stark verbreitet war.

Obwohl das Räuchermittel auf Lentizellenwucherungen und auf nicht verheilte, mechanisch beschädigte Knollenstellen einen schädlichen Einfluss ausübte, wurde die Lagerfähigkeit der behandelten Knollen nicht beeinträchtigt. Um jedoch das gute Ausschen der Knollen zu erhalten, wird empfohlen, die Behandlung von unreifen oder frisch beschädigten Kartoffeln zu vermeiden, da sonst Verfärbung vorkommen kann.

Es kann daraus geschlossen werden, dass 2-Aminobutan in der Praxis angewendet werden kann, um bei Pflanzgut in einem für geschlossene Luftumwälzung eingerichteten Lagergebäude eine sehr gute Bekämpfung von Phoma-Knollenfäule und Tüpfelfleckigkeit und eine teilweise Bekämpfung von Silberschorf zu erzielen.

Résumé

Fumigation de pommes de terre en tas avec le 2-aminobutane pour lutter contre la gangrène, l'oosporiose et la gale argentée

En octobre 1970, on a introduit, par recirculation de l'air, du 2-aminobutane gazeux dans un tas de 35 tonnes de tubercules de la variété *Pentland Crown*, tas contenant également des échantillons des variétés *King Edward* et *Redskin*. Après fumigation, quelques-uns des échantillons traités ont été transférés dans d'autres tas de 35 tonnes, auxquels différents traitements de conservation furent appliqués (tableau 1).

Le 2-aminobutane fut appliqué à la dose de 200 mg/kg avec une vitesse de ventilation de 2,8 m^3 min^{-1} $tonne^{-1}$ pendant environ 50 minutes. Cette application fut suivie d'une recirculation ultérieure de l'air pendant 2 heures. Les mensurations des concentrations de gaz dans les intervalles d'air entre les tubercules durant la fumigation ont révélé une répartition probablement uniforme du 2-aminobutane au travers du tas. Ce que les analyses de résidus ont confirmé (tableau 2).

Les résultats de cotation de maladies après cinq mois de conservation montrent une bonne efficacité du 2-aminobutane contre la gangrène (tableau 3) et l'oosporiose (tableau 4). Ces maladies sont réellement absentes sur les échantillons du tas fumigé, et leur présence est négligeable dans les échantillons fumigés transférés et soumis à d'autres traitements de conservation. L'action des différents traitements de conservation sur l'importance de la gangrène et de l'oosporiose chez les tubercules non fumigés est faible par comparaison avec les effects marqués de la fumigation. La fumigation réduit le niveau d'infection de gale argentée (tableau 5), mais à un degré moindre que pour la gangrène et l'oosporiose, spécialement lorsque la température de conservation est élevée et lorsque le champignon est abondant dans les tubercules au moment du remplissage des box.

Bien que la substance fumigée a un effect néfaste sur les lenticelles proliférées, de même que sur les surfaces endommagées par les machines et non cicatrisées, le pouvoir de conservation des tubercules traités est sauvegardé. Toutefois, pour préserver l'aspect des tubercules, il est recommandé de ne pas traiter des tubercules non mûrs ou fraîchement endommagés, sinon des décolorations peuvent apparaître.

Les auteurs concluent que l'utilisation du 2-aminobutane peut entrer dans la pratique commerciale pour combattre efficacement la gangrène et l'oosporiose, partiellement la gale argentée, sur des plants de pomme de terre stockés dans un bâtiment permettant la circulation de l'air en circuit fermé.

References

Graham, D. C. & G. A. Hamilton, 1970. Control of potato gangrene and skin spot diseases by fumigation of tubers with sec-butylamine. *Nature, Lond.* 227: 297–298.

Graham, D. C., G. A. Hamilton, C. E. Quinn, & A. D. Ruthven, 1973. Use of 2-aminobutane as a fumigant for control of gangrene, skin spot and silver scurf diseases of potato tubers. *Potato Res.* 16: 109–125.

Hide, G. A., J. M. Hirst & G. A. Salt, 1968. Methods of measuring the prevalence of pathogenic fungi on potato tubers. *Ann. appl. Biol.* 62: 309–318.

Nagdy, G. A. & A. E. W. Boyd, Susceptibility of potato varieties to skin spot *(Oospora pustulans)* in relation to the structure of the skin and eye. *Eur. Potato J.* 8: 200–214.

Nash, M. J. & J. H. Lennard, 1970. Cool storage of potatoes in farm-sized stores. 1. Forced-draught ventilation using cold outside air. *Potato Res.* 13: 7–28.

Nash, M. J. & J. H. Lennard, 1973. Forced-draught ventilation with cold outside air compared with recirculation of artificially cooled air. *Potato Res.* 16: 47–52.

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Identification of *Agrobacterium gypsophilae* Strains NCPPB 179 and NCPPB 1948 as *Erwinia herbicola*

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On the basis of 56 characters, the organisms known as *Agrobacterium gypsophilae* NCPPB 179 and NCPPB 1948 are identified as strains of *Erwinia herbicola*. These strains did not cause gall formation when inoculated into four species of *Gypsophila* or into *Lychnis chalcedonica*.

In 1934, Brown (3) described a bacterium which she isolated from galls found at the unions of stock and scion on plants of *Gypsophila paniculata* in the United States. The organism, named *Bacterium gypsophilae*, was a gram-negative rod which was motile by means of several bipolar flagella. It formed yellow colonies on beef infusion agar, was facultatively anaerobic, liquefied gelatin slowly, reduced nitrate to nitrite, did not produce indole, did not hydrolyze starch, and produced acid from glucose, sucrose, maltose, mannitol, and glycerol, but not from lactose. Brown stated that the organism caused galls when inoculated into healthy plants of *G. paniculata* and that it was also pathogenic to species of *Silene, Dianthus, Lychnis*, and *Saponaria*. Because of its purported capability to cause plant galls, it was transferred to the genus *Agrobacterium* by Starr and Weiss (16).

Maas Geesteranus and Barendsen (14) isolated a similar organism from galls on roses in the Netherlands and stated that pathogenicity tests showed the organism to be the cause of the galls. We isolated another yellow-pigmented, gram-negative, rod-shaped bacterium from galls at the graft union of *G. paniculata* cultivar (cv) Bristol Fairy, growing in a local nursery.

De Ley et al. (6) studied the deoxyribonucleic acid base composition of many isolates of agrobacteria and pointed out that the organism in the National Collection of Plant Pathogenic Bacteria (NCPPB) named *Agrobacterium gypsophilae* NCPPB 179 had a mol% G+C value significantly lower than that of *A. tumefaciens, A. rubi*, or *A. rhizogenes*. In addition, they found that NCPPB 179 was not pathogenic to *Datura stramonium* or *Lycopersicon esculentum*. Because many of the phenotypic characters of NCPPB 179 also did not agree with those of other members of the genus *Agrobacterium* but corresponded more closely with those of members of the family *Enterobacteriaceae*, De Ley (5) suggested that the organism might be a member of that family, and, because of its yellow pigmentation, it might be related to *Erwinia herbicola*. White (17) drew attention to the fact that the organism from rose galls (NCPPB 1948) might be related to the genus *Erwinia*.

In view of these possibilities, we carried out studies to establish the identity of the organisms NCPPB 179 and NCPPB 1948.

MATERIALS AND METHODS

Bacterial strains. *A. gypsophilae* NCPPB 179 was originally *B. gypsophilae* 179 in the collection of the late W. J. Dowson, who had received a culture of this strain from M. P. Starr. According to the Catalogue of Strains (1) of the American Type Culture (ATCC), *A. gypsophilae* Dowson 179 is the same organism as *A. gypsophilae* ATCC 13329, the latter having been passed by N. A. Brown to H. J. Conn and thence to M. P. Starr, who deposited it in the ATCC. ATCC 13329 (=NCPPB 179) has been designated (12) as the type strain of *A. gypsophilae*. As mentioned above, NCPPB 1948 is the organism isolated from roses in the Netherlands (14). Also included for comparison in the pathogenicity tests were *Erwinia milletiae* strain JMI isolated from galls on *Milletia floribunda* in Japan and received from M. Goto, the organism isolated by us from *G. paniculata*, and two other strains of *E. herbicola*, which had been isolated by us from plant material. The characters of *E. milletiae* are given by Graham and Hodgkiss (10) and correspond with those of *E. herbicola;* the organism from *G. paniculata* and the two isolates from other plant material were identified as *E. herbicola* as described by Graham and Hodgkiss (10).

Methods. For pathogenicity tests, healthy plants of *G. paniculata* cv Bristol Fairy were selected at a local nursery, potted into pots (20-cm diameter), and kept

in a greenhouse at 16 to 21 C. When the stems of the plants were about 15 cm high, all stems on one plant were inoculated with isolate NCPPB 179, another plant was inoculated with isolate NCPPB 1948, a third was inoculated with our own isolate from galls, and the fourth was left as a control. The inoculation was done by placing a large loopful of bacteria from a 24-h nutrient agar culture (grown at 25 C) on the graft union and pricking the organism into the tissue with a sterile needle. The control plant had sterile water pricked into the graft union instead of bacteria. Soil was then drawn up around the inoculation points so that the graft unions were not exposed. *Gypsophila elegans, G. pacifica, G. repens,* and *Lychnis chalcedonica* (grown from seed) were inoculated with each of the six organisms mentioned above in the same way at their stem bases when they were about 10 cm high. Five plants were inoculated with each isolate, and five were kept as controls.

All plants were covered with plastic bags after inoculation to prevent quick drying at the inoculation points; the bags were removed after 48 h. Temperatures were kept at 16 to 21 C and, although the humidity was not controlled, it was kept high by regularly watering the plants and spraying down the floor and benches of the greenhouse. The plants were grown for 15 weeks and examined periodically for gall formation.

The methods used to characterize NCPPB 179 and NCPPB 1948 were the same as those described by Graham and Hodgkiss (10). Flagella were observed on cells grown on nutrient agar for 48 h at 20 C with a JEOL JEM100B electron microscope using an accelerating voltage of 80 kV and an initial magnification of ×10,000; the cells were negatively stained with a 1% wt/vol potassium phosphotungstate solution. Observations were also made on cells selected for motility using the Craigie tube technique. Sloppy agar (1% wt/vol peptone [Oxoid] + 0.5% [wt/vol] meat extract [Lemco] + 0.3% [wt/vol] agar) was placed to a depth of about 5 cm in a screw-capped vials containing Craigie tubes. Inoculations were made onto the agar surface in the Craigie tube; cultures were incubated at 25 C, and, when growth reached the surface of the agar outside the tube (usually about 72 h), a loopful was transferred from the surface to a tube of ordinary nutrient agar.

RESULTS AND DISCUSSION

Although the inoculated *Gypsophila* and *Lychnis* plants were kept for 15 weeks, there was no evidence of gall formation or any other pathological response. Knösel (11) also found a strain of *A. gypsophilae* that was not pathogenic to *Gypsophila paniculata.* Examination of plants of *G. paniculata* cv Bristol Fairy grown at several nurseries in Scotland showed that gall-like formation often developed at the graft unions; these galls appeared to be callus tissue.

A. gypsophilae NCPPB 179 and NCPPB 1948 contained gram-negative, motile rods which gave the same reactions in the following tests. Positive results were obtained for: fermentative metabolism of glucose in the O/F (Hugh and Leifson) test; acid production from glucose, maltose, trehalose, rhamnose, xylose, sucrose, arabinose, mannitol, inositol, glycerol, mannose, and salicin; liquefaction of gelatin; utilization of citrate, malonate, acetate, mucate, galacturonate, and tartrate; production of a yellow, nondiffusible pigment; production of nitrite from nitrate; catalase production; growth in 5% NaCl broth, and *p*-nitrophenyl-β-D-galactopyranoside hydrolysis. Negative results were obtained for: gas from glucose; acid production from lactose, raffinose, erythritol, adonitol, dulcitol, and inulin; production of indole; pectate gel liquefaction; hydrolysis of starch; rotting of potato slices; production of oxidase, lysine, and ornithine decarboxylases, and arginine dihydrolase, urease, and extracellular deoxyribonuclease; and growth in 10% NaCl broth and in KCN. The two organisms gave different reactions in the tests shown in Table 1.

Examination of these two strains under the electron microscope showed that most cells were nonflagellated, but both contained some cells with only a single lateral flagellum and a very few cells showed several lateral flagella. After selection of motile organisms using Craigie tubes, both strains yielded cultures containing many cells bearing several lateral flagella (Fig. 1). Brown (3) stated that the flagellation of *Bacterium gypsophilae* was bipolar, but, as Graham and Hodgkiss pointed out (10), it is easy to make mistakes with the light microscope.

The phenotypic characters described above are similar to those of the yellow-pigmented, fermentative rods from various habitats identified by Graham and Hodgkiss (10) as *Erwinia*

TABLE 1. *Characters by which strains NCPPB 179 and NCPPB 1948 differ*[a]

Character	NCPPB 179	NCPPB 1948
Symplasmata	–	+
Biconvex bodies	–	+
Growth at 37 C	+	–
Acid on ethanol agar	–	+
Methyl red test	–	+
Voges-Proskauer test	–	+
H_2S production	+	–
Lipolysis	–	+
Gluconate test	–	+
Phenylalanine deaminase	+	–

[a] See Graham and Hodgkiss (10) for test methods.

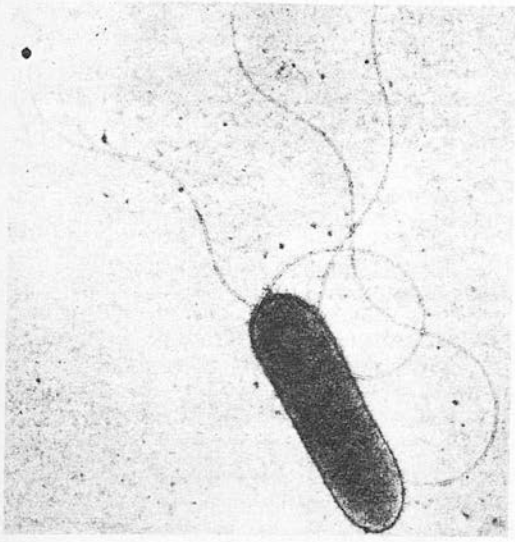

FIG. 1. *Agrobacterium gypsophilae strain NCPPB 179; 48-h culture on nutrient agar at 20 C. Negatively stained with potassium phosphotungstate.* × *14,000.*

herbicola. The differences between NCPPB 179 and NCPPB 1948 are well within the range of variability of this group of organisms as described by Graham and Hodgkiss (10). In slide-agglutination tests, both organisms agglutinated with antiserum (diluted 1:10 with 0.8% [wt/vol] saline) prepared against a strain of *E. herbicola* (serotype 2; see Muraschi et al. [15]). This helped to confirm the identity of the organisms.

There is no reason to doubt the authenticity of *A. gypsophilae* NCPPB 179. Its history is known, and its characters correspond with those given by Brown (3) except with regard to flagellation (a character which is open to misinterpretation) and plant pathogenicity. Although NCPPB 179 did not cause gall formation in our tests, this does not necessarily mean that NCPPB 179 is a bacterium different from Brown's organism because it is possible that NCPPB 179 was once pathogenic and has lost virulence in culture. Furthermore, it seems unlikely that cultures of the original organism have become replaced by a different bacterium having the characteristics of the original organism excepting plant pathogenicity and flagellation. It is noteworthy that NCPPB 1948 and *E. milletiae* JMI were also nonpathogenic to *Gypsophila* and *Lychnis,* but pathogenicity was not tested on the hosts from which these strains were first isolated.

It is concluded that *Agrobacterium gypsophilae* NCPPB 179 and NCPPB 1948 are strains of *Erwinia herbicola* (Löhnis) Dye.

In the *Index Bergeyana* (4), the authorities for the name *E. herbicola* are given as "(Geilinger) Dye," but Löhnis (13) used the name *Bacterium herbicola* for this organism in 1911, thus antedating Geilinger's use of the specific epithet *herbicola* by some 10 years. The name *Bacterium herbicola* was used by Löhnis in a Table for the identification of bacteria, and there is no doubt that the organism referred to is *Bacterium herbicola aureum* Düggeli (7), the name from which the epithet *herbicola* was derived. It might be argued that the name *B. herbicola* was only incidentally mentioned by Löhnis and therefore was not validly published (Rule 12c, International Code of Nomenclature of Bacteria [8]), but this does not seem convincing because Löhnis obviously intended the name to be used for organisms identified by using the Table, and hence the name cannot be regarded as having been merely incidentally mentioned. The name *Bacillus herbicola* was used as long ago as 1905 by Beijerinck (2), but the context in which the name was used makes it clear that this was an incidental mention, and thus this name was not validly published (see Ewing and Fife [9] for a translation of the relevant part of Beijerinck's paper). Ewing and Fife (9) have recently proposed that *Erwinia herbicola* be transferred to the genus *Enterobacter* as *Enterobacter agglomerans* (Beijerinck) Ewing and Fife. However, we prefer to retain the organism in the genus *Erwinia* as *Erwinia herbicola* until comparative studies of all genera presently placed in the family *Enterobacteriaceae* clarify the taxonomy and nomenclature of these organisms.

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REPRINT REQUESTS

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LITERATURE CITED

1. American Type Culture Collection. 1972. Catalogue of strains, 10th ed. American Type Culture Collection, Rockville, Md.

2. Beijerinck, M. W., and A. Rant. 1905. Wundreiz Parasitismus und Gummiflus bei den Amydaleen. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. **11**:366-375.
3. Brown, N. A. 1934. A gall similar to crown gall, produced on *Gypsophila* by a new bacterium. J. Agr. Res. **48**:1099-1112.
4. Buchanan, R. E., J. G. Holt, and E. F. Lessel (ed). 1966. Index bergeyana. The Williams & Wilkins Co., Baltimore, Md.
5. De Ley, J. 1968. DNA base composition and hybridization in the taxonomy of phytopathogenic bacteria. Annu. Rev. Phytopathol. **6**:63-90.
6. De Ley, J., M. Bernaerts, A. Rassel, and J. Guilmot. 1966. Approach to an improved taxonomy of the genus *Agrobacterium*. J. Gen. Microbiol. **43**:7-17.
7. Düggeli, M. 1904. Die Bacterien flora gesunder Samen und daraus gezogener Keimpflanzchen. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. **12**:602-614; **13**:56-63, 198-207.
8. Editorial Board of the Judicial Commission of the International Committee on Nomenclature of Bacteria (ed). 1966. International code of nomenclature of bacteria. Int. J. Syst. Bacteriol. **16**:459-490.
9. Ewing, W. H., and M. A. Fife. 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the Herbicola-Lathyri bacteria). Int. J. Syst. Bacteriol. **22**:4-11.
10. Graham, D. C., and W. Hodgkiss. 1967. Identity of gram negative, yellow pigmented fermentative bacteria isolated from plants and animals. J. Appl. Bacteriol. **30**:175-189.
11. Knösel, D. 1962. Untersuchugen an sternbildenden Bakterien. Arb. Landwirtsch. Hochsch. Hohenheim **15**:1.
12. Lessel, E. F. 1962. Bacterial type cultures of the American Type Culture Collection. Int. Bull. Bacteriol. Nomencl. Taxon. **12**:71-88.
13. Löhnis, F. 1911. Landwirtschaftlich-bakteriologishes Prakticum. Gebruder Borntraeger, Berlin.
14. Maas Geesteranus, H. P., and H. Barendsen. 1966. Host plants of *Agrobacterium gypsophilae*. Neth. J. Plant Pathol. **72**:231-232.
15. Muraschi, T. F., M. Friend, and D. Bolles 1965. *Erwinia*-like microorganisms isolated from animal and human hosts. Appl. Microbiol. **13**:128-31.
16. Starr, M. P., and J. E. Weiss. 1943. Growth of phytopathogenic bacteria in a synthetic asparagin medium. Phytopathology **33**:314-318.
17. White, L. O. 1972. The taxonomy of the crown-gall organism *Agrobacterium tumefaciens* and its relationship to rhizobia and other agrobacteria. J. Gen. Microbiol. **72**:565-574.

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Control of potato gangrene by fumigation of tubers with 2-aminobutane after periods of storage

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Zusammenfassung, Résumé p. 414

Summary

When potato stocks infected with the gangrene fungus (*Phoma exigua* var. *foveata*) are graded after a period of storage, apparently healthy tubers can subsequently develop gangrene. Fumigation with 2-aminobutane (at a dose of 200 mg/kg) of such tubers, taken from 7 stocks which had been in storage several months (but were not showing visible sprouts), gave variable but significant reductions in the amount of gangrene that subsequently developed, provided treatment was done soon after grading. Fumigation of tubers showing visible gangrene lesions did not stop the lesions from spreading.

Analyses of tubers for 2-aminobutane showed that residues in the peel and flesh were much lower than in tubers treated soon after harvest. It is suggested that the poorer gangrene control given by fumigation after storage resulted from lower absorption and poorer skin penetration by the 2-aminobutane.

Introduction

Fumigation of seed tubers with 2-aminobutane gives very good control of potato gangrene (caused by *Phoma exigua* var. *foveata*), when treatment is done within 14 days of harvesting (Graham et al., 1973). However when the period between harvest and treatment lengthens to 30 days, the efficiency of the treatment diminishes. Graham et al. (1973) suggested that the decline in effectiveness resulted from the skin and damaged areas becoming increasingly impervious to the gas as a consequence of physico-chemical changes in the skin after lifting. In addition it was suggested that the fungus might have penetrated to depths in the tuber which the 2-aminobutane could not reach in sufficient concentration to kill the organism.

It is well known that the efficiency of other chemical treatments in controlling several tuber diseases declines quite rapidly after harvest. For instance, treatment with organo-mercurial dipping solution should be done within 3 days of harvest to get best results for control of gangrene (Graham et al., 1973). However, the necessity to treat tubers so soon after lifting presents serious practical problems especially when chemicals that may only be applied to seed are used, as separation of seed and ware (table) potatoes has to be done quickly, very often while growers are still lifting crops. Many growers prefer to store the mixture of seed and ware, separating them up to several months later. In Scotland, much of this grading is done during January

and February, by which time gangrene infection in any of the stocks has usually become manifest. An important characteristic of gangrene is that latent infection associated with the tuber becomes activated as a result of tubers being damaged during grading and further handling. Preliminary experiments in which tubers bearing visible lesions were fumigated showed that treatment did not stop the lesions from spreading, but it was thought possible that fumigation might prevent development of new lesions, particularly as the gas might be able to penetrate damaged areas and kill the fungus there. Experiments described here were carried out in 1971, 1973 and 1974 to determine if any benefit could be derived from fumigation after tubers had been in storage at least 3 months.

Materials and methods

Stocks of 7 cultivars containing varying amounts of visibly gangrenous tubers were obtained from commercial sources. The tubers had been lifted during the months of September or October and stored either in bulk or in pallet boxes. A quantity of apparently healthy tubers (for treatments described below) was carefully hand-picked from the farm store to minimise further damage; the remainder was brought to the laboratory in sacks and graded either with a rubber spool-grader or a bare-wire reciprocating riddle within 2 days of receipt. Care was taken to see all visibly diseased tubers were removed. Fumigation with 2-aminobutane was carried out with tubers placed in trays in a 5-tonne chamber, according to the method described by Graham et al. (1973), at a dosage of 200 mg/kg. The various treatments are given in Table 1. After treatment tubers were stored in trays in a chitting house where temperatures varied between 9–15 °C. All disease assessments were made in the middle of April. These assessments were largely made by visual diagnosis, but representative samples were checked for gangrene by cultural methods.

Residue analyses for 2-aminobutane in the skin and flesh of tubers from 3 stocks were done by the methods given in Graham et al. (1973). Analyses were made to see how residue levels differed between treatments; how residues compared with those obtained when treatments were done soon after lifting; and to see if there was any relationship between the distribution of 2-aminobutane in the peel and flesh and the level of disease control.

Results

Details of the various treatments and their effects on development of gangrene are summarized in Table 1.

Results of 2-aminobutane residue analyses are given in Table 2.

Table 1. Effects of different treatments on development of gangrene after tubers had been stored.

Cultivar[1]	Date of grading[2]	Grading method[3]	Number of days elapsed between grading and treatment[4]	Number of tubers examined[5]	Percentage gangrene[6]
1. Pentland Crown	1–3–71	hand[7]	1	505	5.9
		hand	NF*	462	5.4
		riddle[8]	1	1658	2.4
		riddle	8	1568	3.6
		riddle	NF	1554	7.1
2. Pentland Dell	1–3–73	hand	1	718	2.5
		hand	NF	864	2.7
		riddle	1	1657	1.6
		riddle	7	1517	0.7
		riddle	NF	2221	1.3
		spool[9]	1	1639	1.7
		spool	8	1139	2.5
		spool	NF	1856	2.5
3. Bintje	11–1–73	hand	1	456	3.1
		hand	NF	435	13.1
		riddle	1	1283	8.9
		riddle	7	1543	13.6
		riddle	NF	1643	50.0
4. Doon Star	11–1–73	riddle	1	609	1.0
		riddle	7	778	2.6
		riddle	NF	635	47.9
5. King Edward	11–1–73	riddle	1	645	0.9
		riddle	7	704	1.0
		riddle	NF	649	45.6
6. Maris Peer	19–2–74	hand	1	533	2.3
		hand	NF	510	8.0
		riddle	1	1072	5.0
		riddle	7	800	6.3
		riddle	NF	1080	10.7
		spool	1	1322	2.5
		spool	7	983	6.3
		spool	NF	976	10.8
7. Pentland Squire	20–2–74	hand	1	422	5.9
		hand	NF	395	13.6
		riddle	1	626	8.2
		riddle	7	726	11.7
		riddle	NF	728	18.4
		spool	1	655	10.8
		spool	7	626	13.6
		spool	NF	700	18.0

*NF = Not fumigated – *Nicht begast – Pas fumigé*

[1] *Sorte – Cultivar;* [2] *Datum der Sortierung – Date du calibrage;* [3] *Sortiermethode – Méthode de calibrage;* [4] *Anzahl Tage zwischen Sortierung und Behandlung – Nombre de jours entre le calibrage et le traitement;* [5] *Anzahl untersuchter Knollen – Nombre de tubercules examinés;* [6] *% Phoma-Fäule – Pourcentage gangrène;* [7] *Hand – Main;* [8] *Sieb – Crible;* [9] *Walzen – Rouleau.*

Tabelle 1. Einfluss verschiedener Behandlungen auf die Entwicklung der Phoma-Krankheit an Knollen nach der Einlagerung.
Tableau 1. Effets de différents traitements sur le développement de la gangrène après conservation des tubercules.

Table 2. Residues of 2-aminobutane in treated tubers.

Cultivar[1]	Grading method[2]	Number of days elapsed between grading and treatments[3]	Residue in flesh[4] (mg/kg)	Residue in peel[5] (mg/kg)	Calculated residue in whole tuber[6] (mg/kg)	Residue removed by peeling[7] (%)
1. Bintje	hand[8]	1	1	37	5	75
	riddle[9]	1	7	71	14	54
	riddle	7	5	41	9	50
2. Maris Peer	hand	1	5	109	22	79
	riddle	1	10	184	31	70
	riddle	7	7	82	15	59
	spool[10]	1	8	173	30	74
	spool	7	5	67	13	66
3. Pentland Squire	hand	1	3	89	13	80
	riddle	1	12	189	30	66
	riddle	7	2	57	9	84
	spool	1	7	139	22	75
	spool	7	4	75	10	68

[1] *Sorte – Cultivar;* [2] *Sortiermethode – Méthode de calibrage;* [3] *Anzahl Tage zwischen Sortierung und Behandlung – Nombre de jours entre calibrage et traitements;* [4] *Rückstand im Fleisch – Résidu dans la chair;* [5] *Rückstand in der Schale – Résidu dans la peau;* [6] *Errechneter Rückstand in der ganzen Knolle – Résidu calculé sur le tubercule entier;* [7] *Beim Schälen entfernter Rückstand – Résidu enlevé par épluchage*
[8] *Hand – Main;* [9] *Sieb – Crible;* [10] *Walzen – Rouleau*

Tabelle 2. Rückstände von 2-Aminobutan in behandelten Knollen.
Tableau 2. Résidus de 2 aminobutane dans les tubercules traités.

Discussion

In general, the results achieved in controlling gangrene in stored tubers when treatment was done one day after grading were not as good as those obtained by fumigating with 2-aminobutane within 14 days of harvest. Control was even lower when treatment was done 7–8 days after grading. It is noteworthy, however, that in some cases very good results were obtained, as in the experiments with the cultivars Doon Star and King Edward, and in several experiments the level of control was such as to be commercially worthwhile, especially if the stock was particularly valuable or where particularly high levels of gangrene might be expected to develop. The results also incidentally illustrate how mechanical grading induces development of new gangrene lesions, and that as much gangrene develops irrespective of whether grading is by spool grader or by bare wire riddle, even though spool grading generally causes less visible tuber damage. Somewhat surprisingly, fumigation of hand-picked tubers having minimal post-storage damage usually gave some control, indicating that the

gaseous 2-aminobutane could still penetrate unbroken tuber skin to a sufficient extent to affect the gangrene fungus. As explained earlier it was originally thought that prevention of gangrene might only occur at damaged areas into which the gas could penetrate.

The residue results did however show that the grading process assisted the subsequent penetration and absorption of 2-aminobutane, as the highest levels in both peel and flesh were obtained when treatment was done one day after grading. Residues in tubers treated 7 days after grading (Table 2) were always lower than those in tubers treated one day after grading, indicating that tubers had become less pervious to gaseous 2-aminobutane during the period of storage in trays after grading, possibly through a combination of wound healing and changes in skin permeability. This would probably account for the fact that these treatments were less efficient in controlling gangrene. Residue levels in both the peel and flesh of hand-picked and graded stored tubers, shown in Table 2, proved to be substantially lower than in tubers treated soon after lifting (compare with data in Table 2 given in Graham et al. (1973)). Likewise the calculated whole tuber residues were much below the theoretical value of 200 mg/kg, which could account for the generally lower level of disease control as compared with treatments done soon after harvest. Finally, the analyses did not show any correlation between the ratio of fumigant residues in peel and flesh and the method of grading, or the subsequent levels of disease control.

Fumigations of stocks in January and February are now being carried out in Scotland on a commercial scale. Treatment at this time of year has no deleterious effect on subsequent growth of the tubers or on the yield; full details of growth and yield experiments will appear elsewhere. It should be noted, however, that none of the treated tubers showed visible sprouts; fumigation of sprouted tubers is not recommended as the fumigant may damage the young growth.

Zusammenfassung

Bekämpfung der Phoma-Krankheit an Kartoffeln durch Begasung von Knollen mit 2-Aminobutan nach verschiedenen Lagerungsperioden

Wenn mit dem Phoma-Pilz (*Phoma exigua* var. *foveata*) infizierte Kartoffeln nach einer Lagerungsperiode sortiert werden, kann sich bei anscheinend gesunden Knollen nachträglich Phoma entwickeln. Es wird angenommen, dass dies weitgehend auf Beschädigungen zurückgeht, die die latente, mit der Knolle verbundene Infektion aktivieren. Viele Pflanzer finden es vorteilhaft, ein Gemisch von Speise- und Pflanzkartoffeln mehrere Monate einzulagern, bevor sie es sortieren, mit dem Risiko, dass sich nach der Sortierung häufiger Phoma entwickelt. Vorversuche, in denen Knollen mit sichtbaren Läsionen mit 2-Aminobutan (Dosierung 200 mg/kg) begast wurden, zeigen, dass die Behandlung die Weiterausbreitung der Schäden nicht hinderte. Es wurde jedoch als möglich angenommen, dass durch die Begasung die Entwicklung neuer Fäulnisstellen vermieden werden könnte, besonders weil das Gas vermutlich in die beschädigte Zone, von der aus sich oftmals Phoma entwickelt, eindringt. Während der Jahre 1971, 1973 und 1974 wurden 7 Phoma-infizierte Posten, die mindestens 3 Monate eingelagert waren (deren Knollen noch keine sichtbare Keime zeigten), mit der Hand, mit Schwingsieb- und mit Walzensortier-

gerät (spool grader) sortiert. Die Knollen wurden dann entweder einen oder 7–8 Tage später mit einer Dosis von 200 mg/kg 2-Aminobutan begast. Die Ergebnisse sind in Tabelle 1 zusammengefasst. Sie zeigen, dass – obwohl das Ausmass der Krankheitsbekämpfung unterschiedlich und oft niedriger war als wenn die Knollen kurz nach der Ernte behandelt wurden – doch eine genügende Wirkung erreicht wurde, damit sich eine Behandlung kommerziell lohnt, besonders wenn hochwertige Bestände befallen sind.

Analysen von Knollen, die 7 Tage nach dem Sortieren mit 2-Aminobutan behandelt wurden, zeigen, dass die Rückstände in Schale und Fleisch viel kleiner waren als in Knollen, die kurz nach dem Sortieren behandelt wurden (Tabelle 2). Es wird angenommen, dass die schwächere Phoma-Bekämpfung nach der Lagerung auf die niedrigere Absorption und das schwächere Eindringen durch die Schale von 2-Aminobutan zurückzuführen ist.

Résumé

Contrôle de la gangrène des pommes de terre par fumigation des tubercules avec 2-aminobutane après des périodes de stockage

Quand des stocks de pommes de terre infectées par le champignon de la gangrène (*Phoma exigua* var. *foveata*) sont calibrés après une période de stockage, les tubercules apparemment sains peuvent subséquemment développer de la gangrène. On croit que ceci résulte en grande partie des blessures qui activent l'infection latente provenant des tubercules.

Beaucoup de cultivateurs trouvent avantageux de conserver tubercules de consommation et plants en mélange pendant plusieurs mois avant de les séparer avec le risque conséquent du développement de plus de gangrène aprês le calibrage. Des essais préliminaires dans lesquels des tubercules porteurs de lésions visibles sont soumis à la fumigation avec 2-aminobutane à la dose de 200 mg/kg ont montré que le traitement ne stoppe pas l'extension des lésions. Cependant il est possible que la fumigation puisse prévenir le développement de nouvelles lésions, particulièrement parce que le gaz pénètrerait dans les surfaces endommagées, à partir desquelles la gangrène se développe souvent. Au cours des années 1971, 1973 et 1974, 7 stocks infectés de gangrène ont été mis en conservation pendant au moins 3 mois (mais les tubercules ne montraient pas des germes), puis calibrés à la main, par crible alternatif et par calibreurs à rouleaux. Les tubercules ont été alors soumis à la fumigation avec 2-aminobutane à une dose de 200 mg/kg, soit un jour, soit 7–8 jours plus tard. Les résultats sont résumés dans le tableau 1. Ils montrent bien que le degré de contrôle de la maladie est variable et souvent moindre quand les tubercules sont traités aussitôt après la récolte, on atteint un contrôle suffisant que pour rendre le traitement commercialement valable, spécialement quand des stocks de haute valeur sont infectés.

Les analyses de tubercules pour le 2-aminobutane ont montré que les résidus dans la peau et la chair sont beaucoup plus faibles que chez les tubercules traités aussitôt après la récolte (tableau 2). Il est supposé que le résultat plus faible de lutte contre la gangrène provient d'une plus faible absorption et d'une plus faible pénétration du 2-aminobutane dans la peau.

Reference

Graham, D. C., G. A. Hamilton, C. E. Quinn & A. D. Ruthven, 1973. Use of 2-aminobutane as a fumigant for control of gangrene, skin spot and silver scurf diseases of potatoes. *Potato Res.* 16: 109–125.

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Potential Spread of Erwinia spp. in Aerosols

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ABSTRACT

Bacterial aerosols were generated in a chamber by simulated raindrops falling on potato stems infected with *Erwinia carotovora* var. *atroseptica*. Viable propagules moved readily with slow air streams and remained suspended in the air for at least 60-90 minutes. Phytopathology 65:739-741

Additional key words: dissemination, sprinkler irrigation.

Soft rots of vegetables and other plants caused by *Erwinia* spp. are important problems everywhere. The pathogens are reported to be soil-borne, and contamination of fleshy organs directly from the soil has been believed to be the primary source of inoculum. Newer evidence, however, indicates that *Erwinia* spp. do not survive well in soil (5, 8,), at least in temperate climates. Potatoes freed from *Erwinia* have become recontaminated in isolated areas (6), and other crops not vegetatively propagated are often infected. This raises important questions about inoculum sources. Splash dispersal may spread bacteria and wind-blown rain has been implicated in the spread of some bacterial pathogens for longer distances (4). The possibility exists that aerosols generated by rain or overhead sprinklers could be responsible for long-distance spread of soft-rotting bacteria. Southey and Harper (9) reported the survival of artificially generated *Erwinia amylovora* aerosols for 3 hours, but no other known information on the occurrence and potential importance of aerosols as a means of spread has been reported for bacterial plant pathogens. This report describes laboratory experiments to determine if aerosols can be generated from potato tissues infected with *Erwinia carotovora* var. *atroseptica* (van Hall) Dye by impaction of water drops.

MATERIALS AND METHODS.—Water drops ranging from 2 mm to 5 mm in diameter were dropped down a 15.24-cm diameter plastic tube from a height of 7.62 m onto infected potato stems (cultivar Pentland Javelin) placed in a flat tray (containing approximately 3.81 cm of sterile soil) located in a stainless steel tank large enough to contain any splash droplets (Fig. 1). Water drops 2 mm in diameter falling from 7.62 m reach terminal velocity while 5 mm drops reach 94% of terminal velocity (7). Humidified air (approximately 90-95% RH and 10.6 C) was drawn into the tank then out through a wind tunnel made of 30.5-cm diameter aluminum tubing 2.74 m long by a variable-speed fan located at the exit end of the tube. Ports located at 30.5-cm intervals along the tube facilitated sampling the moving air. The distance from the target site to the end of the tunnel was approximately 4.1 m.

A Casella bacteria slit sampler (C. F. Casella and Co. Ltd., London, England) connected with a rubber tube to the sampling ports was used to sample air moving through the tube at various distances from the target stems. Bacteria were deposited on Stewart's double-layer pectate medium (10) in petri dishes which were subsequently incubated at 26 C for 48 hours, then examined for *Erwinia* colonies. Pectolytic *Erwinia* spp. form characteristic colonies on this medium which can be readily distinguished from other pectolytic bacteria such as *Pseudomonas* spp., *Bacillus* spp., and *Flavobacterium* spp. which may be associated with potato stems.

RESULTS.—*Generation of aerosols by simulated raindrops.*—Water drops 5 mm in diameter fell on six stem sections approximately 15.2 cm long at the rate of 960 drops per minute for 5 minutes. Air was drawn through the system at the rate of approximately 22.9 m/minute. The air was sampled approximately 4.6 m from the stems at the volume of 30 liters per minute for 10-minute periods beginning at the time drops began to fall, and ending 15 minutes after drop delivery had ceased. The system was checked for contamination by following the above procedure using healthy stems immediately before the experiment with infected material.

Large numbers (approximately 600 colonies per plate) of airborne *Erwinia* were collected during the first 10 minutes of the experiment and some (four colonies per plate) were collected for at least 10 minutes after water drops had ceased. This suggests that bacterial aerosols that move with a slow air stream were generated by the simulated raindrops. The experiment was repeated using a slower rate of simulated rainfall (130 drops per minute) with similar results.

Relation of raindrop size and number to aerosol generation.—Since 5 mm water drops approach the maximum size of raindrops (1) an experiment was designed to study the effect of drop size and number on aerosol generation. Drops 2, 3, 4, and 5 mm in diameter bombarded infected potato stems with the air flow and humidification systems shut off. Immediately after the

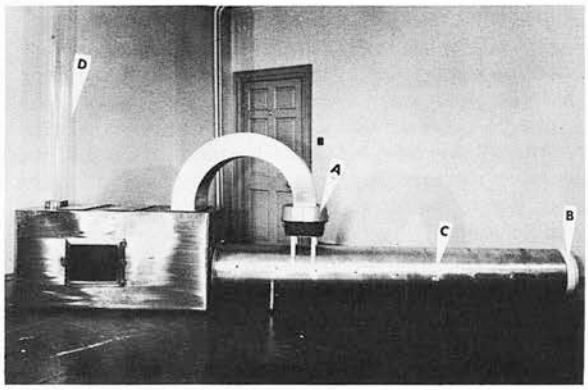

Fig. 1. Apparatus used to study bacterial (*Erwinia carotovora* var. *atroseptica*) aerosol formation by simulated raindrops striking infected potato stems. Legend: A=humidifier, B=variable-speed fan, C=port for sampling air stream, D=plastic tube through which water drops were delivered. Dimensions of the main chamber were: height 76.2 cm, width 55.9 cm, and length 152.4 cm.

TABLE 1. Relation between size and number of simulated raindrops and generation of bacterial (*Erwinia* sp.) aerosols from blackleg-infected potato stems

Drop size (mm)	Number of drops	Number of *Erwinia* colonies/150 liters of air[a]
2	1	0
	5	1
	10	2
	25	8
	50	7
	100	17
	Total	35
3	1	1
	5	15
	10	0
	25	34
	50	33
	100	57
	Total	140
4	1	32
	5	0
	10	18
	25	42
	50	84
	100	104
	Total	280
5	1	43
	5	30
	10	49
	25	53
	50	32
	100	49
	Total	256

[a]Air was sampled at the rate of 30 liters/minute for 5 minutes at a point approximately 4.0 m from the target stems. Air sampling began immediately after the designated number of drops had struck the diseased stems.

required number of drops had struck the stems, the systems were turned on and air (92% RH) was drawn through the apparatus at 18.3-21.3 m per minute. Air was sampled for 5 minutes (150 liters of air sampled) at a point approximately 4.0 m away from the target area. Control plates were exposed between treatments, and plate counts were adjusted to compensate for any bacteria remaining in the system between treatments.

The results (Table 1) show that detectable numbers of bacteria became airborne after the stems were struck by as few as five drops 2 mm in diameter or one drop 3 mm in diameter. Bacterial numbers increased as drop size and number increased up to 4 mm. There was no further increase in bacterial numbers using drops 5 mm in diameter.

Suspension time of bacterial aerosols.—An experiment was designed to determine the length of time aerosols generated by water drops remained suspended in the system. Infected potato stems were bombarded with 5 mm diameter water drops falling at the rate of 1,000 drops per minute for 5 minutes. During this time the air flow and humidification systems were turned off, but immediately after the simulated rainfall ceased, the fan and humidifier were started and air was pulled through the system at a velocity of 21-22.9 m per minute. The air was sampled for a period of 30 seconds at a point approximately 4.0 m from the target area then the air flow was immediately stopped. The process was repeated at 15, 30, and 60 minutes after the rainfall ceased. At 90, 120, and 150 minutes the air was sampled for 2 minutes and at 180 minutes the air was sampled for 5 minutes.

Detectable numbers of viable particles remained suspended in the system for at least 60 minutes after simulated rainfall ceased. Bacterial numbers decreased from approximately 300-400 colonies per plate in the initial sample to 1 colony per plate in the 60-minute sample. No bacteria were detected in the 90-, 120-, 150-, and 180-minute air samples.

The actual number of aerosol particles containing bacteria per unit volume of air could not be accurately determined since sampling was anisokinetic; i.e., the air speed in the sampling tube was much greater than in the wind tunnel.

DISCUSSION.—The formation of airborne droplets containing viable bacteria which remain suspended for a relatively long period of time and move in slow air streams adds a new dimension to the epidemiology of soft rots and related diseases caused by *Erwinia carotovora* var. *atroseptica* and similar organisms. Aerosols generated by rain showers or sprinkler irrigation systems in arid areas may move considerable distances on light breezes under the appropriate conditions. Because some

particles remained suspended in still air for 60 – 90 minutes they must be small; i.e., in the 4- to 8-μm range (3). Once airborne, propagules of this size may remain suspended for prolonged periods in moving air.

Formation of *Erwinia* aerosols in this same size range probably occurs in the field as indicated by limited sampling in Colorado.

The susceptibility of *Erwinia* cells to desiccation, radiation, open-air factor (2), etc. will determine the potential role of aerosols in the epidemiology of soft rot bacteria, but nothing is yet known about these aspects.

LITERATURE CITED

1. BEST, A. C. 1950. The size distribution of raindrops. Quart. J. Roy. Meteorol. Soc. 76:16-36.
2. DRUETT, H. A. 1973. The open air factor. Pages 141-149 *in* J. H. Ph. Hers, and K. C. Winkler, eds. Airborne transmission and airborne infection. 6th International Symposium on Aerobiology. Halsted Press, John Wiley & Sons, New York, 610 p.
3. DUGUID, M. B. 1946. The size and duration of air-carriage of respiratory droplets and droplet nuclei. J. Hyg. (Camb.) 44:471-479.
4. FAULWETTER, R. C. 1917. Wind blown rain, a factor in disease dissemination. J. Agric. Res. 10:639-648.
5. GRAHAM, D. C. 1958. Overwintering of soft rot bacteria in Scottish soils. Nature(Lond.)181:61.
6. GRAHAM, D. C., and J. L. HARDIE. 1971. Prospects for the control of potato blackleg disease by the use of stem cuttings. Proc. 6th Br. Insectic. Fungic. Conf. 1:219-224.
7. GREGORY, P. H., E. J. GUTHRIE, and M. E. BUNCE. 1959. Experiments on splash dispersal of fungus spores. J. Gen. Microbiol. 20:328-354.
8. LOGAN, C. 1969. The survival of the potato blackleg pathogen overwinter. Rec. Agric. Res. Minist. Agric. N. Ire. 17:115-121.
9. SOUTHEY, R. F. W., and G. J. HARPER. 1971. The survival of Erwinia amylovora in air-borne particles: Tests in the laboratory and in the open air. J. Appl. Bact. 34:547-556.
10. STEWART, D. J. 1962. A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. Nature(Lond.)195:1023.

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Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967-74

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Zusammenfassung, Résumé p. 17

Summary

Propagation of potato stocks from stem cuttings has produced material almost entirely free from infection with *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica*; during 1967-74, very few infected plants or tubers were found on the nuclear stock farm. When infection occurred, *E. carotovora* var. *carotovora* was the organism most often isolated, whereas in ordinary commercial stocks *E. carotovora* var. *atroseptica* predominates. In 1973 and 1974 dipterous insects caught at a nearby dump of decomposing vegetable matter were contaminated with *E. carotovora* var. *carotovora* several weeks before infection was found on potato stems. Serotypes of *E. carotovora* var. *carotovora* isolated from insects and infected plants were very often identical, providing compelling evidence that the source of the organisms was the dump, from which contaminated insects dispersed and subsequently transferred organisms to the crop. The origin of infections with *E. carotovora* var. *atroseptica* remains unknown.

Introduction

In recent years, research on a number of potato tuber diseases has focused attention on the importance of the mother tuber as the major source of pathogenic micro-organisms attacking both the plants grown from the mother tubers and their progeny. Potato blackleg, caused by *Erwinia carotovora* var. *atroseptica* (subsequently referred to as *E. atroseptica*), and tuber soft rot, which can be caused by both *E. carotovora* var. *carotovora* (subsequently referred to as *E. carotovora*) and *E. atroseptica*, belong to this group of diseases: Graham & Hardie (1971) summarized the evidence that soft rot coliform bacteria are not inhabitants of Scottish soils. Isolations made from blackleg infected stems in Scotland almost always yield *E. atroseptica* (Graham & Dowson, 1960) whereas both *E. carotovora* and *E. atroseptica* were

isolated from all of 48 Scottish commercial tuber stocks, usually in the ratio of 80% *E. atroseptica* to 20% *E. carotovora* (Perombelon, 1973). Much of this infection is latent - tubers show no visible signs of disease throughout the storage period. The important fungal diseases, skin spot and gangrene, also appear to be largely stock-borne. Because the pathogens that cause these diseases are associated with the tuber, new generations of tubers produced by propagation from stem cuttings are much less likely to be infected. However, as soft rot coliform bacteria can spread into potato stems from mother tubers, cuttings must be tested bacteriologically and found free from infection before propagation (Graham & Hardie, 1971). Aiming at a general reduction in the incidence of blackleg, soft rot and other latent tuber diseases in potatoes, the Department of Agriculture and Fisheries for Scotland (DAFS) attempted to obtain nuclear seed stocks from tested stem cuttings using existing virus-free stocks in 1967. The early results were so promising that it was decided to produce all virus-tested nuclear stocks in this way with the eventual objective of replacing all potato stocks in Scotland with material originating from stem cuttings. Nuclear stocks grown from stem cuttings are referred to as VTSC (i.e. derived from virus-tested stem cuttings) and constitute the highest grade in the DAFS Seed Potato Certification Scheme. To avoid re-infection as far as possible the nuclear VTSC stocks have been raised on an upland farm called Ingraston, some 30 km south-west of Edinburgh, in an area where very few other commercial crops of potatoes are grown. Before 1967 Ingraston farm itself had never grown potatoes. Tubers originally derived from stem cuttings are released to specialist VTSC growers for further multiplication who, in turn, pass their produce to growers of lower grades. The background to the VTSC project, the production of stem cuttings and their propagation, methods for isolating and testing for pathogenic organisms, possible transmission of infection by insects and some early experiences relating to re-infection, are discussed by Graham & Hardie (1971).

This paper describes the successful production of VTSC stocks at Ingraston in relation to soft rot coliform bacterial infection. It also discusses recurrence of infection - incidentally widening the general understanding of the epidemiology of soft rot diseases caused by *Erwinia* spp. Bearing in mind the distances separating the Ingraston crop from other commercial crops (usually at least 50 km), the occurrence of infection at Ingraston despite strict hygienic precautions, and reports in the literature of association of insects with soft rot coliforms, led to a bacteriological study of insects in the Ingraston environment. To establish more precisely the identity and thus any epidemiological relationship between soft rot coliforms from sources in and around Ingraston, serotyping was carried out, as is commonly done in studies on the epidemiology of other genera of the Enterobacteriaceae.

Bacteriological and serological methods used in the epidemiological study

Method of isolation and identification of Erwinia spp.

Plant material was suspended in a little sterile water and a loopful plated on Mac-

Table 1. Organisms used to prepare antisera.

Organism[1]	Reference number[2]	Source plant[3]	Country of origin[4]
E. carotovora var. *carotovora*	NCPPB 438	Iris	USA
	NCPPB 671	Carnegiea	USA
	NCPPB 1742	Brassica	Brazil
	NCPPB 547	Persea	Israel
	NCPPB 1745	Brassica	Japan
	NCPPB 312	potato tuber[5]	Denmark
	NCPPB 66	tobacco[6]	Uganda
	NCPPB 550	tobacco	USA
E. carotovora var. *atroseptica*	G 110	potato stem[7]	Scotland

[1] *Erreger - Organisme;* [2] *Bezugsnummer - Numéro de référence de l'antisérum;* [3] *Wirtspflanze - Plante infectante;* [4] *Herkunftsland - Pays d'origine;* [5] *Kartoffelknolle - Tubercule de pomme de terre;* [6] *Tabak - Tabac;* [7] *Kartoffelstengel - Tige de pomme de terre*

Tabelle 1. Erreger, die zur Herstellung der Antiseren verwendet wurden.
Tableau 1. Organismes utilisés pour préparer l'antisérum.

Conkey pectate medium (Stewart, 1962), incubated at 26 °C, and pectolytic colonies characteristic of soft rot coliform bacteria transferred to nutrient agar slopes. Organisms from the slopes were replated on nutrient agar and single colonies transferred to nutrient agar slopes to ensure culture purity. On occasion the MacConkey pectate plates were heavily overgrown with other organisms making it impossible to select discrete colonies. In these circumstances a loopful of colony growth was transferred to a potato tuber slice and incubated on damp filter paper in a Petri dish at 26 ° for 24 h. Uninoculated slices were kept as controls. The inoculated potato slice usually rotted, acting as an enrichment medium for the soft rot bacteria. Isolations were then made from the rotted tissue as before.

When insects were tested for soft rot coliforms, individuals or several insects bulked together were crushed in a drop of sterile water between sterile microscope slides. A large loopful of fluid from the slide was plated on MacConkey pectate; pectolytic colonies were picked from the plates and purified as previously described.

Soft rot coliform organisms were identified to specific and sub-specific level by the methods described by Graham (1972).

Serotyping

Isolates of *E. carotovora* obtained in 1971-74 were serotyped using a range of antisera prepared against the organisms listed in Table 1. Lazar (1972) has shown that there are a large number of serotypes amongst the soft rot coliform group and

Table 2. Serotypes of *E. carotovora* var. *carotovora* found on plant material and insects at Ingraston farm.

Antiserum	Serotype									
	1	2	3	4	5	6	7	8	9	10
NCPPB 438	−	+	+	−	−	−	−	+	−	−
NCPPB 671	−	−	−	−	−	−	+	−	−	−
NCPPB 1742	+	+	−	+	−	−	−	−	−	−
NCPPB 547	−	−	+	−	+	−	−	−	−	−
NCPPB 1745	+	+	−	−	−	−	−	−	+	−
NCPPB 312	−	+	−	−	−	−	−	−	−	+
NCPPB 66	−	−	−	−	−	−	−	−	−	−
NCPPB 550	−	−	−	−	−	−	−	−	−	−
G 110	+	+	−	−	−	−	−	−	+	−

Tabelle 2. Serotypen von *E. carotovora* var. *carotovora*, die an Pflanzen und Insekten in Ingraston gefunden wurden.
Tableau 2. Sérotypes d'*E. carotovora* var. *carotovora* trouvés sur le matériel végétal et les insectes à la ferme d'Ingraston.

most of the antisera were received from Dr Lazar. Antisera against NCPPB 66 and NCPPB 312 were prepared by us according to the method described by Graham (1963). All antisera had titres lying between 1:1000 and 1:2000.

Serotyping was carried out using a simple slide agglutination test. A loopful of a 24-48 h culture grown on nutrient agar was emulsified in a drop of tap water on a slide, 1 drop of antiserum (diluted 1:10 with 0.85 % saline) was added and mixed by rocking. Agglutination reactions were read within 2-3 min of adding antiserum.

During the years 1971-74 inclusive, soft rot coliform infections, mainly caused by *E. carotovora* were found on VTSC potato material and from other sources at Ingraston, except in 1972 when infection with *E. atroseptica* predominated in the growing crop. Table 2 gives details of the serotypes of *E. carotovora* found over the period 1971-74. Isolates of *E. atroseptica* were not serotyped.

Although the fact that 2 organisms from different sources prove to be the same serotype does not necessarily imply an origin in common, it is believed that in the rather isolated environment of Ingraston, identity of serotype suggests strongly that the organisms are of common origin.

Epidemiological studies at Ingraston Farm relating mainly to occurrence of Erwinia carotovora var. carotovora on plants, tubers and insects

Investigations were made both during the growing season and on potato tubers in storage; these are described chronologically.

Growing seasons 1967-70

Although mother tubers were known to be the main source of soft rot coliform contamination of daughter tubers, one could not foresee how successful the stem cutting project might be in the short or long term. There were many conceivable ways in which re-infection might occur, such as by contaminated machinery (an obvious example), but also through other avenues so far unproven or unknown. However, every attempt was made to ensure that nuclear stocks were propagated under the best hygienic conditions attainable. Over the period 1967-70 inclusive about 260 000 plants were grown at Ingraston; all were rigorously inspected but only 1 plant (cv. Redskin) was found infected, in this case with *E. atroseptica*. The source of infection was never traced. Eighty-four other samples of suspect plants were examined bacteriologically but found uninfected.

Storage periods 1967-70

Tubers of all stocks stored at Ingraston were carefully inspected throughout the storage periods for soft rot, heel end necrosis or other doubtful symptoms. Out of 1636 stocks 11 contained soft-rotted and other suspect tubers which were subjected to a bacteriological examination. Of these 6 did not yield soft rot coliform bacteria, but 1 stock gave *E. atroseptica* and 4 stocks gave tubers infected with *E. carotovora*.

Growing season 1971

In late August and early September, 21 soft rot infections distributed at random throughout an area containing about 4000 plants were discovered on stems of tested first-year stem cuttings. From the more severe symptoms on two plants with stem lesions it was clear that they had been infected some weeks previously and tubers on these plants showed extensive internal rots caused by organisms identified as *E. atroseptica*. Both plants were next to wheel tracks suggesting infection had been introduced by machinery. A nearby plot, containing plants grown from tubers two years away form the stem cutting stage, contained several blackleg-infected plants which might have been the source. The remaining infections on the stem cuttings were greenish or brownish wet rots either on stems above soil level or at exposed leaf scars, and apparently had been established recently because lesions had not spread extensively. The plants suffered wind damage and because of prolonged wet weather, the haulms were senescent and rotting: the rotting material was attracting a large number of insects including many fruit flies (*Drosophila* sp.) and some wasps (*Vespa* sp.); 12 *Drosophila* , 3 *Vespa* and 5 other unidentified insects were collected and 2 - a *Drosophila* sp. and a *Vespa* sp. - yielded soft rot coliform bacteria. Isolations were made from 21 infected stems; 16 isolates of soft rot coliforms were obtained but were identified only to generic level and were discarded before further tests were made. However, two isolates from the fruit fly and the one from the wasp were identified to specific level and found to be *E. carotovora*. Further isolations were made from first-year stem cutting stems in mid-October after the crop had been lifted. This could be done because diquat had been used as a haulm destroyer,

and as the weather remained wet, it was quite easy to find rotting stems at that time. From 18 stems 18 *E. carotovora* and 2 *E. atroseptica* isolates were obtained, whereas no *Erwinia* spp. were isolated from another 6 stems. This was an unexpected result, because, as mentioned earlier, the organism almost invariably isolated from rotting stems in commercial crops in Scotland is *E. atroseptica*. Pathogenicity tests on potato stems, by the method of Graham & Dowson (1960), showed that 6 isolates from stem cuttings, 1 from *Drosophila* sp. and 1 from *Vespa* sp. did not cause typical blackleg disease, but a localized soft rot, showing that the organisms were pathologically distinct from *E. atroseptica*. The total number of *E. carotovora* isolates collected from the stems and insects was 21, of which 13 were serotyped; 6 stems yielded serotype 1; 3 stems serotype 6; 1 stem serotype 7; 1 *Drosophila* sp., serotypes 6 and 8, and 1 *Vespa* sp., serotype 1. It was concluded that insects in the crop were contaminated with *E. carotovora*, and were probably spreading infection, but it was impossible to say whether the insects had introduced the bacteria, or whether the insects had become contaminated with the organisms while feeding on already infected stems.

Storage period 1971-72

Twenty stocks out of 501 contained tubers affected with soft rot or heel end necrosis; altogether 62 such tubers were found. Seven stocks were infected with *E. carotovora* alone and 8 with *E. carotovora* and *E. atroseptica* together. These results do not correspond with the situation in ordinary commercial stocks, in which infections with *E. atroseptica* predominate. However the amount of disease in the stocks should not be overestimated from these data for only 62 diseased tubers were found out of a total production of some 80 tonnes (although, of course, other tubers could be carrying latent infection). It is also noteworthy that the number of plants constituting a stock grown in earlier years was generally smaller than in 1971 or later years.

Of the isolates of *E. carotovora* 4 were serotyped: 2 were of serotype 1, 1 of serotype 6, and 1 of serotype 7. All these serotypes were found in the growing crop in 1971.

Growing season 1972

Because insects might have been responsible for introducing infection into first year stem cutting material, it was decided to investigate whether insects in the immediate environment of the crop were contaminated with soft rot coliforms. An electrically driven insect trap was made by mounting a 30-cm diameter Vent-Axia fan vertically on a stand 1.5 m high. A muslin bag was attached beneath the fan, protected from wind by a metal cylinder which fitted round the bag and was fixed to the underside of the Vent-Axia mounting. The fan was operated continuously in the crop from mid-June until mid-September. Insects were caught dry in the bag which was changed twice a week; the collections were sorted and individuals identified to the order and sometimes the genus to which they belonged, and tested for soft rot

coliforms. Live insects were also swept from vegetation immediately surrounding the potato field, and taken to the laboratory for plating within 24 h. Additionally, special attention was paid to a turnip crop growing close to the potatoes.

Between 14 June and 14 September 1494 insects comprising 633 Diptera, 216 Coleoptera, 152 Hymenoptera, 253 Hemiptera, 153 *Thrips* spp., 28 Lepidoptera and 49 others were tested. Of these 909 were caught in the trap, 242 were swept from the turnips, and the rest were obtained from other sites around the crop. Only 4 insects, all of which were caught alive in the turnip crop, yielded soft rot coliform bacteria, in every case *E. carotovora*: a hymenopteran caught on 27 July, a dipteran caught on 24 August, another dipteran on 7 September, and a hymenopteran on 14 September.

No soft rot infections of stems similar to those in 1971 occurred on the first-year stem cuttings (though typical blackleg was seen sporadically in plots 2, 3 and 4 years beyond the stem cutting stage and the causal organisms identified as *E. atroseptica*). Furthermore, as the insects carrying the bacteria came from the turnips, these plants were scrutinized carefully. On 31 July, 1 turnip was found with extensive soft rot beginning at the petiole bases; this rot was found to be caused by *E. carotovora*. On 3 August, another similarly infected plant was found, and by the second week in September about a quarter of the crop (some 2000 plants) was affected. Rotting always began on the above ground parts of the plants, suggesting some kind of airborne transmission. Three isolates from insects and 3 from the plants were all found to be serotype 8.

The observations again showed that insects were contaminated with and possibly transmitting soft rot coliforms, but their significance as vectors could not be judged as it was impossible to say whether the insects brought infection into the crop or had become contaminated when feeding on already infected plants.

Live insects always yielded many more bacteria than those from the insect trap; it was concluded that the latter method of collection was unsuitable, probably because the air stream constantly blowing through the bag dried up the insects and apparently killed many of the associated bacteria.

The haulms of the VTSC crop were destroyed with sodium chlorate sprays during August and September, and because of the dry weather the haulm bleached and dried quickly. Eleven samples of stems taken at random from the crop and tested did not yield any soft rot coliforms.

Storage period 1972-73

Three stocks out of 367 contained soft rotted tubers from which *E. carotovora* was obtained. None were serotyped as no *E. carotovora* had been isolated from the 1972 growing crop. However, it was later realized that it would have been of value to know whether the organisms from the tubers were of the same serotype as those found on insects and turnips in the 1972 growing season, but the cultures had been destroyed.

Fig. 1. Site of waste dump where insects were found contaminated with soft rot coliform bacteria in 1973 and 1974. The arrow marked 1973 indicates the precise location where the insects were caught; the arrow marked 1971 shows the area in the field in the background where infected first year-stem cuttings were found in 1971.

Abb. 1. Lage des Abfallhaufens an dem 1973 und 1974 Insekten kontaminiert mit coliformen Nassfäulebakterien gefunden wurden. Der Pfeil, markiert mit 1973, gibt die genaue Stelle an, wo die Insekten gefangen wurden; der andere Pfeil, markiert mit 1971, zeigt im Hintergrund die Stelle des Feldes wo 1971 infizierte Pflanzen aus dem Nachbau der Stengelstecklinge gefunden wurden.

Fig. 1. Emplacement du dépôt de détritus où les insectes ont été trouvés contaminés par les bactéries coliformes de la pourriture molle en 1973 et 1974. La flèche marqué 1973 indique la localisation précise où les insectes ont été capturés; la flèche marquée 1971 montre la partie du champ à l'arrière-plan où des boutures de tige de première année ont été trouvées infectées en 1971.

Growing season 1973

Occurrence of *E. carotovora* infections on stem cuttings and an annual crop, like turnips, on the farm continued to suggest that there might be a source of this organism nearby, from which insects could become contaminated and transmit infection to the crop.

Accordingly, the environment surrounding Ingraston was searched on 24 July, and at any site where dead leaves or other plant debris were found, insects were collected and taken to the laboratory for identification (usually to generic level) and bacteriological examination. One site near a disused railway line consisted of an

area roughly 50 m × 30 m of decomposing vegetable matter, just outside the confines of the farm. Special attention was paid to it, as it was only some 120 m south-west of the place where VTSC stem cuttings were infected in 1971 with *E. carotovora* and 150 m north-west of the 1973 VTSC crop. The site, illustrated in Fig. 1, was heavily overgrown with weeds, but quantities of garden waste had evidently been deposited repeatedly, and 4 potato plants were found growing close together at one place. It was learned later that both farm and garden waste had been deposited there for many years. Sweepings for insects were made in the immediate vicinity of the potato plants; and 15 potato tubers which had already formed on the plants were tested and found infected with *E. carotovora* serotype 1. Insects were again collected on 1 August, 5 September and 16 October for testing for soft rot coliforms, some separately and others bulked according to the genus. From plates on which bulked material had been streaked, several colonies (usually 4-6) were selected for identification and serotyping.

From each sampling from one part of the site, no more than about 4 m square,

Table 3. Serotypes of *E. carotovora* var. *carotovora* found associated with insects and potato stems in 1973.

Source[1]	Date of collection[2]	Serotype[3]
1. Bacteria isolated from insects[4]		
Leptocera sp. (11 bulked[5])	24. 7.73	3+4+5
Leptocera sp. (15 bulked)	24. 7.73	5
Fannia sp. (1 insect[6])	1. 8.73	3
Unidentified dipteran[7] (3 bulked)	5. 9.73	3
Fannia sp. (1 insect)	16.10.73	3
2. Bacteria isolated from potato stems[8]		
Potato stem[9] (Up-to-date)	10. 8.73	2
	10. 8.73	2
	10. 8.73	2
	10. 8.73	2
Potato petiole[10]	10. 8.73	3
Potato stem (Majestic)	15. 8.73	4
	15. 8.73	5

[1] *Quelle - Source;* [2] *Samlungsdatum - Date d'observation;* [3] *Serotyp - Sérotype;* [4] *Bakterien isoliert von Insekten - Bactéries isolées des insectes;* [5] *Insgesamt - Amas;* [6] *Insekt - Insecte;* [7] *Nicht identifizierte Dipteren - Diptères non identifiés;* [8] *Bakterienisolate von Kartoffelstengeln - Bactéries isolées des tiges de pomme de terre;* [9] *Kartoffelstengel - Tige de pomme de terre;* [10] *Kartoffelblattstiel - Pétiole de pomme de terre*

Tabelle 3. Serotypen von *E. carotovora* var. *carotovora*, die an Insekten und Kartoffelstengeln 1973 gefunden wurden.

Tableau 3. Sérotypes d'*E. carotovora* var. *carotovora* trouvés associés aux insectes et aux tiges de pomme de terre en 1973.

flies (*Leptocera* spp., *Fannia* spp. and unidentified dipterans) yielded *E. carotovora*. Out of 133 insects tested from this area, at least 5 must have been carrying the bacteria (it was impossible to tell the exact number because of bulking), whereas 205 insects from 11 other areas around Ingraston were not.

Soft rots well above ground level were discovered on 10 August on stems of 19 plants in a plot of cv. Up-to-Date. Another plant had a blackened petiole scar and samples were examined bacteriologically. On 15 August, in a plot of cv. Majestic, mechanically damaged stems with fluid exuding from them were sampled. *E. carotovora* was isolated from both lots of material; results of serotyping organisms from insects and plants are summarized in Table 3. These results showed that all 3 serotypes associated with the insects in July were subsequently found infecting potato plants in August, 17 and 22 days after insects had been caught at the waste dump and found contaminated. Although another serotype found on plants was not associated with the insects tested, the results pointed to the insects as a source of infection on the potato plants. Furthermore, insects found at the waste dump were still carrying soft rot coliforms in mid-October, long after the haulms had been destroyed with sodium chlorate in August and September, and the crop harvested. (As in 1972, 1973 was a dry harvesting season and haulms dried and bleached quickly.) It is known that insects of the genera *Leptocera* and *Fannia* are 'dirty' feeders, breeding in organic waste, and observations in the field revealed that these insects visited the crop, and were particularly attracted by fluid exuding from damaged stems.

On 16 October, the small part of the waste dump that yielded contaminated insects was dug over to a depth of about ½ m looking for any particular plant remains, such as potato tubers, which might have been a source of bacteria. All that was found was well decomposed vegetable matter except for a bulb of Dutch iris (*Iris hollandica*), which was infected with *E. carotovora* serotype 6, a serotype last found on potatoes during the 1971-72 storage period. The source of the bacteria associated with the insects from this site therefore remained undiscovered.

Storage period 1973-74

Only 3 stocks out of 340 contained soft rotted tubers. Two were infected with *E. carotovora*. Five isolates of *E. carotovora* (3 from 1 stock and 2 from another) were serotyped. All were serotype 1, a serotype found in the growing crop and on an insect in 1971, but not in the growing crop or on insects in 1973, although it was found on potato tubers from the plants growing in the dump in 1973.

During 1967-73, tuber infections were always found in stocks 2 or more years beyond the stem cutting stage of propagation, except in 1971 when tubers from 2 plants of first year stem cuttings were infected with *E. atroseptica*.

Growing season 1974

Insects were collected at the site of the rubbish dump at roughly fortnightly intervals, from 17 June to 9 October, and on 3 occasions in a nearby turnip crop. Over

Table 4. Serotypes of *E. carotovora* var. *carotovora* found associated with insects and potato stems in 1974.

Source[1]	Date of collection[2]	Serotype[3]
1. Bacteria isolated from insects[4]		
Leptocera sp. (4 bulked[5])	18. 7.74	3
Leptocera sp. (1 insect[6])	31. 7.74	2
Leptocera sp. (1 insect)	19. 8.74	8
Unidentified dipteran[7] (1 insect)	18. 9.74	6
2. Bacteria isolated from potato stems[8]		
Potato stems[9]	5. 9.74	2
	5. 9.74	2
	5. 9.74	2
	5. 9.74	2
	18. 9.74	6
	18. 9.74	8
	18. 9.74	8
	18. 9.74	6
	18. 9.74	9
	18. 9.74	2
	9.10.74	10
	9.10.74	10
	9.10.74	3
	9.10.74	6
	9.10.74	6

[1-9] *Siehe Tabelle 3 - Voir le tableau 3*

Tabelle 4. Serotypen von *E. carotovora* var. *carotovora*, die 1974 an Insekten und Kartoffelstengeln gefunden wurden.
Tableau 4. Sérotypes d'*E. carotovora* var. *carotovora* trouvés associés aux insectes et aux tiges de pomme de terre en 1974.

this period 639 insects (sometimes bulked according to genus, others as individuals) were tested bacteriologically. Four lots were found infected with *E. carotovora*; 3 from the same limited area of the dump as in 1973 and 1 from the turnip crop. No soft rot stem infections were seen in the growing potato crop or the turnips. Potato haulm was destroyed with a dinoseb spray during August and September, but some stems remained green, although they eventually rotted. After harvest, 64 of these stems (some of which included first year stem cuttings) were taken at random from the field on 3 occasions and tested bacteriologically; 15 yielded *E. carotovora*. Results of serotyping organisms from insects and stems are detailed in Table 4. These results show that all the serotypes found on the insects were also found on the potato stems left in the field after lifting, together with two additional serotypes (9 and 10) found only on potato stems. Serotype 2, found on potato haulm but not on insects in 1973, was found on an insect caught at the dump and on stems

Table 5. Number of plants grown, and number affected by blackleg in the period 1967-74.

Year[1]	Total number of plants grown[2]	Number affected by blackleg[3]
1967	1 000	0
1968	9 000	0
1969	120 000	1
1970	130 000	0
1971	170 000	15
1072	170 000	42
1973	150 000	12
1974	100 000	3

[1] *Jahr - Année;* [2] *Gesamtzahl der angebauten Pflanzen - Nombre total de plantes ayant végété;* [3] *Zahl der Pflanzen mit Schwarzbeinigkeit - Nombre de plantes affectées par la jambe noire*

Tabelle 5. Gesamtzahl der Pflanzen und Anzahl der Pflanzen mit Schwarzbeinigkeitssymptomen in den Jahren 1967-1974.
Tableau 5. Nombre de plantes ayant végété et nombre de plantes affectées par la jambe noire au cours de la période 1967-1974.

in 1974. It is concluded that despite the finding of serotypes 9 and 10 only on stems, the observations again strongly suggest that infections with *E. carotovora* in the crop originated from insects dispersing from the dump of waste nearby.

Infection of potato plants and stored tubers at Ingraston with Erwinia carotovora var. atroseptica

Over the years, a very small number of potato plants showing typical blackleg symptoms occurred in crops at Ingraston, mostly in 1972. Details are given in Table 5. All affected plants were tested bacteriologically and found infected with *E. atroseptica*. Infections were often associated with certain clones and except for 1 case were found in plants grown from tubers at least 2 years beyond the stem cutting stage. In 1972, when 42 blackleg infected plants were found, 26 were confined to 4 plots (each of a different cultivar) out of 367 plots. It is noteworthy that the 1972 infections followed the finding of 13 stocks carrying *E. atroseptica* infection in the 1971-72 storage period as discussed below.

Tubers in storage were also found infected with *E. atroseptica*; details of the number of stocks in store and the number of stocks found infected are given in Table 6. Compared with the total number of stocks grown, very few were infected with this organism and indeed notably fewer than with *E. carotovora*. In each stock only a few tubers (usually 2 or 3) were affected with soft rot or heel end necrosis.

The origin of the infections with *E. atroseptica* remains unknown; the organism was never found in association with insects caught in and around Ingraston. It might be argued that the bacteriological procedure used for testing the cuttings failed to detect latent infection. Whereas this is possible, the figures show that since 1967,

Table 6. Occurrence of *E. atroseptica* in stocks in storage over the period 1967-74.

Year[1]	Total number of stocks[2]	Number of stocks infected with *E. atroseptica*[3]
1967-70	1636	1
1971-72	501	13*
1972-73	367	0
1973-74	340	1

* 8 of the stocks were also infected with *E. carotovora* - *8 dieser Kartoffelpartien waren auch mit E. carotovora infiziert* - *8 de ces lots étaient aussi infectés par E. carotovora*

[1] *Jahr* - *Année*; [2] *Gesamtzahl der Kartoffelpartien* - *Nombre total de lots*; [3] *Anzahl der mit E. atroseptica infizierten Kartoffelpartien* - *Nombre de lots infectés par E. atroseptica*

Tabelle 6. Auftreten von *E. atroseptica* in Kartoffelpartien im Lager während der Jahre 1967-1974.

Tableau 6. Observations d'*E. atroseptica* sur des lots en stockage au cours de la période 1967-1974.

only 2 of some 20 000 tested cuttings have been found infected with soft rot coliforms; in both instances, *E. carotovora* was isolated.

Discussion

Production of stem cuttings and multiplication of progeny at Ingraston farm over 8 years has shown that it is possible to produce crops essentially free from infection with soft rot coliform bacteria on a commercial scale. Although reinfections have occurred, the numbers have been very small compared with the total production, and reached very much lower levels of infection that those of ordinary commercial stocks. It is also especially noteworthy that, except in 1972, the organism most frequently found was *E. carotovora* and not *E. atroseptica*. However infections which occurred on VTSC material have provided an opportunity to investigate how soft rot coliforms may survive and spread in the environment. Up till now this has been impossible because alternative ways of survival and transmission have been obscured by the universal infection of ordinary commercial tubers with soft rot coliform bacteria.

Observations that insects can transmit soft rot coliform bacteria are not new and scattered information can be found over the years throughout phytopathological and entomological literature. As long ago as 1926, Leach (1926) showed that the dipterous insect *Hylemyia cilicrura* could transmit *Erwinia* spp. to potatoes, work which suggested that the bacteria persisted throughout the life cycle of the insect. *Hylemyia cilicrura* occurs in Britain, but it was never encountered in this study. There are also reports of dipterans and other insects acting as possible vectors, for instance in relation to soft rot of crucifers and *Erioischia brassicae* (Johnson, 1930; Doane &

Chapman, 1964); and heart rot of celery associated with attacks by the leaf mining insect *Scaptomyza graminum* (Ogilvie et al., 1935). Chiu et al. (1958) reported that various insects, including bees, transmitted soft rot coliform bacteria in cabbage stores, and Tamimi & Banfield (1969) described transmissions of unspecified soft rot organisms from various plants (including potato) to lettuces by species of *Drosophila*. Molina et al. (1974), demonstrated that *Drosophila melanogaster*, contaminated with *E. atroseptica*, transmitted infection to damaged potato stems very efficiently. In a more recent experiment, (Harrison & Graham, unpublished) insects (including *Leptocera* spp. and *Drosophila* spp.) caught at a waste potato dump were placed in a cage containing 5 potato plants with damaged stems. Bacteriological tests showed that 4 out of 5 damaged plants became infected with *E. carotovora* after 3 days. This emphasizes the ease with which these insects can transmit bacteria to damaged parts of plants.

Although the number of insects caught carrying soft rot coliform bacteria at the dump was small compared with those not contaminated, the sample must represent only a fragment of the total population dispersing from the dump throughout the growing season. Nevertheless the observations are also in accord with an epidemiological picture involving relatively few infected insects, otherwise a much larger number of plants at Ingraston probably would have been found infected with *E. carotovora*. Serotyping the isolates of *E. carotovora* indicated that isolates from insects and plants were identical in many cases, from which it may be inferred that the plants were infected by organisms carried by the insects, though other means of transmission cannot be ruled out. It is also clear that there are many serotypes of soft rot coliforms and a larger number of antisera would be of help in further epidemiological research. In this connection it is noteworthy that serotyping did not always indicate a relationship between the bacteria found in the growing crop and those in diseased stored tubers, although not all isolates from tubers were serotyped to conserve the limited supplies of antisera. More studies on these lines are clearly required. Additionally, the stocks in which affected tubers were found during storage were different from the stocks in which infection was found in the growing crop, for all stocks found infected in the growing season are destroyed.

The precise nature of the association of *Erwinia* spp. with *Leptocera* spp., *Drosophila* spp., *Fannia* spp. and other insects is not known, but it may be that insects which live and breed in decaying organic matter, merely become contaminated with *Erwinia* spp. if these happen to be present in the organic waste. Insects including those of the above three genera have been collected from several other heaps of rotting vegetable matter and a manure heap, but soft rot coliforms have not been isolated from them.

It is obvious that if insects are contaminated with *Erwinia* spp., they could act as vectors, for they disperse from their breeding grounds and presumably may travel considerable distances. Yerrington & Warner (1961) showed that *Drosophila melanogaster* could move about 8 km in 24 h; Lempke (1962) found *Fannia canicularis* could travel up to 70 km; and Yates & Lindquist (1952) demonstrated that *Musca*

domestica could move as far as 32 km. Their efficiency as vectors will depend on many other uncertain factors; for instance, the period of survival of soft rot coliforms in association with insects is not known, but tests show it must be at least 24 h.

In summation, the data provide compelling but not conclusive evidence that infections with *E. carotovora* found in VTSC potato plants at Ingraston were established by insects associated with a dump of vegetable matter nearby. Once infection becomes established on plants the population of bacteria could build up on them, especially under favourable conditions (particularly wet weather) and spread could then take place within the crop. Internal spread could occur in several ways, such as by further insect transmission, by contaminated machinery and by rain splash. Establishment of primary foci by insects from outside the crop and probably also transmission within the crop (as insects are unlikely to live in the crop) could not be prevented by spraying with insecticides. The only way to try to ensure freedom from infection is by good hygienic measures, including the disposal of vegetable waste (especially waste potato tubers) by methods other than dumping.

A noteworthy observation is that rotting, but still green, stems found in the field late in the season (and even after the crop had been lifted) in 1971 and 1974 often yielded soft rot coliforms, especially *E. carotovora*. This indicates substantial build-up of stem infection can occur, particularly in wet seasons, and emphasises the need to use an efficient chemical haulm killer. Furthermore, every effort should be made to ensure complete coverage of the crop by the haulm killer as part of general hygienic measures.

This study has not thrown any fresh light on the source of *E. atroseptica*. Perhaps some simple hygienic procedure has been overlooked or contaminated flies reached the crop from far away, but equally there may be as yet undiscovered (or unconfirmed) ways in which soft rot coliform bacteria can spread in the environment. It has, however, highlighted the complexity of the epidemiology of diseases caused by soft rot coliforms and in particular the association of causal organisms with insects.

Acknowledgments

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Zusammenfassung

Erneutes Auftreten von Infektionen mit coliformen Nassfäuleerregern an Kartoffelstengelstecklingen: Eine epidemiologische Studie in der zentralen Saatgutvermehrung in Schottland in den Jahren 1967-74

1967 wurde 30 km von Edinburg entfernt unter des Aussicht des Department of Agriculture and Fisheries for Scotland (DAFS) mit der Produktion von Saatkartoffeln, die von ge-

sundem Nachbau von Stengelstecklingen stammten begonnen. Die Produktion wurde seitdem von spezialisierten Vermehrern als VTSC-Saatgut gesteigert. (VTSC = Nachkommen von virusgetesteten Stengelstecklingen); VTSC ist der höchste Grad im DAFS Anerkennungsschema. Das Ziel dieser Produktionsart ist die Ausschaltung von Krankheitserregern, die im wesentlichen von der Knolle übertragen werden; in diese Gruppe gehören die coliformen Nassfäulebakterien.

Seit 1967 wurde in dem Ausgangssaatgut der DAFS sehr genau das Auftreten von Infektionen mit *Erwinia carotovora* var. *carotovora* (*E. carotovora*) und *Erwinia carotovora* var. *atroseptica* (*E. atroseptica*) überwacht. Während der Jahre 1967-1974 wurde eine kleine Zahl von Stengeln und Knollen infiziert mit diesen Erregern gefunden, aber mit Ausnahme von 1972, war der vorherrschend auftretende Erreger *E. carotovora*, während im gewöhnlichen Konsumanbau in Schottland *E. atroseptica* zu finden war. Infektionen mit *E. carotovora* sind sehr deutlich von typischen Schwarzbeinigkeitssymptomen zu unterscheiden; gewöhnlich findet man grünliche nassfaule Stellen an beschädigten Blättern oder gegen Ende der Wachstumsperiode faulende Stengel.

Im August und September 1971 wurde eine kleine Zahl von Pflanzen mit einem Feld von 4000 Stengelstecklingen hauptsächlich mit *E. carotovora* infiziert gefunden (obwohl auch *E. atroseptica* in geringem Ausmass vorhanden war) und Insekten (einschliesslich *Drosophila* spp. und *Vespa* spp.), die zwischen den Pflanzen gefangen wurden, waren mit *E. carotovora* behaftet. Es konnte nicht festgestellt werden, ob die Pflanzen durch die Insekten infiziert wurden oder umgekehrt, aber um zu prüfen, ob irgendein Zusammenhang zwischen den Organismen der Insekten und der Pflanzen bestand, wurde der Serotyp der Isolate von beiden Herkünften bestimmt. In Tabelle 1 sind die Erreger, die zur Herstellung der Antiseren verwendet wurden aufgeführt und Tabelle 2 zeigt die in den Jahren 1971-1974 gefundenen Serotypen. Es ergab sich, dass die Serotypen, die von den Insekten isoliert wurden, mit den meisten der Isolate der Pflanzen identisch waren. 1972 wurden keine mit *E. carotovora* infizierten Kartoffelstengel gefunden, aber ein nahe gelegener Rübenbestand war infiziert und Insekten, die in diesem Bestand gefangen wurden, enthielten den selben Erreger. Auf dem Feld wurde eine Saugfalle aufgestellt, sie war aber ungeeignet, da die Insekten in ihr schnell starben und austrockneten und nur weinig Bakterien isoliert werden konnten. 1973 wurden auf einem nahegelegenen Gemüseabfallhaufen Dipteren, einschliesslich *Leptocera* spp. und *Fannia* spp., gefangen, die bereits einige Wochen bevor Infektionen mit diesem Erreger an Kartoffelstengeln auftraten, mit *E. carotovora* kontaminiert waren (Fig. 1). Tabelle 3 zeigt die mit dem Erreger kontaminierten Insekten und Pflanzen und die Serotypen. Im wesentlichen ähnlich Ergebnisse wurden 1974 erhalten, obwohl Stegelinfektionen nur an alten Stengeln nach der Ernte beobachtet wurden; die Ergebnisse sind in Tabelle 4 zusammengestellt. Diese Ergebnisse liefern den zwingenden aber nicht endgültigen Beweis, dass eine Infektionsquelle mit *E. carotovora* kontaminierte Insekten von Abfallhaufen sind, die sich auf beschädigten Stengeln, Blättern oder alten Kartoffelstengeln verteilen und sich gleichzeitig davon ernähren.

Während der Jahre 1967-1974, vor allem 1972, wurden einige Kartoffelpflanzen infiziert mit *E. atroseptica* gefunden (Tabelle 5). Diese Infektionen traten im allgemeinen nur an Pflanzen, 2 oder mehr Jahre nach der Bildung der Stengelstecklinge, auf und sie schienen mit bestimmten Klonen verbunden zu sein. So wurden auch nur wenige Partien von Kartoffelknollen, die mit diesem Erreger infiziert waren, im Lager gefunden (Tabelle 6). Der Ursprung von *E. atroseptica* ist unbekannt, es wurden niemals kontaminierte Insekten in Ingraston festgestellt.

Die Ergebnisse erhellen die Bedeutung einfacher Hygienevorschriften, um Reinfektionen mit coliformen Nassfäuleerregern zu verhindern, vor allem durch Vermeidung von Ansammlungen faulender Gemüseabfälle (vor allem Kartoffeln) auf Farmen. Ebenso sollte ein wirksames chemisches Krautabtötungsmittel verwendet werden, um einen möglichen Ausbruch von Stengelinfektionen in der späten Wachstumsperiode zu verhindern. Die Epidemiologie der Nassfäulen verursacht durch *Erwinia* spp. ist noch nicht völlig geklärt und es können noch andere Möglichkeiten für die Verbreitung dieser Erreger bestehen.

Résumé

Réapparition des infections bactériennes de pourriture molle dans les boutures de tiges de pomme de terre: une étude épidémiologique à la Station de production des plants de base en Ecosse pendant les années 1967-74

La production de plants de base, indemnes de maladie, obtenus à partir de boutures de tige, a été commencée en 1967 par le Ministère de l'Agriculture écossais (DAFS), dans une station située à 30 km d'Edimbourg. La descendance a, depuis, été multipliée par des agriculteurs producteurs spécialisés de semences VTSC (boutures de tige testées pour verifier l'absence de virus), la classe VTSC étant la plus élevée dans le plan de certification des semences de pomme de terre du DAFS. Cette pratique a pour but d'éliminer tous les organismes responsables de maladies, qui sont essentiellement transmis par les plants; les bactéries coliformes de la pourriture molle appartiennent à ce groupe.

Depuis 1967, les plants de base du DAFS ont été soigneusement protégés contre les infections d'*Erwinia carotovora* var. *carotovora* (*E. carotovora*) et d'*Erwinia carotovora* var. *atroseptica* (*E. atroseptica*). Au cours de la période 1967-1974, un petit nombre de tiges et de tubercules ont été trouvés infectés par ces organismes, mais exception faite pour l'année 1972, *E. carotovora* était le plus communément présent, alors que dans les lots commerciaux, en Ecosse, c'est *E. atroseptica* qui prédomine. Les infections par *E. cartovora* sont tout à fait distinctes de la jambe noire typique - pourritures molles habituellement verdâtres sur les feuilles au niveau de blessures ou pourritures de tiges sénescentes en fin de période de croissance.

En août et septembre 1971, sur 4000 boutures de première année plantées dans le champ, quelques unes étaient infectées, principalement par *E. carotovora* (*E. atroseptica* n'était présent que sur une petite surface) et les insectes capturés sur les plantes (y compris *Drosophila* spp. et *Vespa* spp.) étaient contaminés par *E. carotovora*. Il était impossible de dire si les plantes avaient été infectées par les insectes ou vice-versa; mais, pour voir s'il y avait une relation, des isolations de chacune des deux sources furent sérotypées.

Les organismes utilisés pour produire l'antisérum sont donnés dans le tableau 1 et les sérotypes trouvés pendant la période 1971-1974 sont détaillés dans le tableau 2. Il a été montré que les sérotypes isolés à partir des insectes étaient identiques à la plupart de ceux isolés à partir des plantes. En 1972, aucune tige de pomme de terre n'était infectée par *E. carotovora*, mais, dans les environs, une récolte de navet était infectée et les insectes vivants capturés dans la parcelle portaient le même organisme. Un piège à insectes a été installé dans le champ, mais ce nu fut pas un succès car les insectes, qui mouraient et se desséchaient trop rapidement, produisaient peu de bactéries. En 1973, des insectes diptères, y compris *Leptocera* spp. et *Fannia* spp., capturés tout près d'un dépôt de détritus de matière végétale, étaient contaminés par *E. carotovora* plusieurs semaines avant que les infections par cet organisme se manifestent sur les tiges de pomme de terre. Les insectes et les plantes portant cet organisme et les sérotypes sont mentionnés dans le tableau 3. Globalement, des résultats similaires ont été obtenus en 1974, bien que les symptômes sur tiges n'apparurent que sur de vieilles tiges après l'arrachage de la récolte; les résultats sont donnés dans le tableau 4.

Sans être une preuve définitive, ces données montrent qu'une source des infections par *E. carotovora* pourraient être des insectes contaminés, associés aux tas de déchets, les insectes disséminant l'organisme après l'avoir prélevé sur les feuilles au niveau des blessures ou sur les tiges sénescentes.

Au cours de la période 1967-1974, quelques plantes de pomme de terre ont été trouvées infectées par *E. atroseptica*, spécialement en 1972, comme le montre le tableau 5. Ces infections n'ont généralement été observées que sur des plantes de deux ou plusieurs années au-delà du stade bouturage et elles avaient tendance à être associées à certains clones. Comparativement, très peu de lots de tubercules de pomme de terre, en magasin, ont été trouvés infec-

tés par cet organisme (tableau 6). L'origine d'*E. atroseptica* reste inconnue; à Ingraston, il n'a jamais été trouvé associé aux insectes.

Ces résultats mettent clairement en évidence l'importance de simples précautions pour éviter la recontamination par les bactéries coliformes de la pourriture molle, spécialement en évitant les accumulations de déchets de matière végétale (en particulier de pommes de terre), dans les fermes. Un défanant efficace pourrait aussi être appliqué pour prévenir de possibles infections de tiges, tard dans la saison. Toutefois, il est aussi mentionné que l'épidémologie des pourritures molles causées par *Erwinia* spp. n'est pas complètement connue, et qu'il peut y avoir d'autres moyens de dissémination de ces organismes dans le milieu ambiant.

References

Chiu, W.-F., C.-S. Yuen & C.-A. Wu, 1958. On the overwintering and dissemination of the soft rot organism *Erwinia aroideae* (Towns) Holland. *Acta phytopath. sinica* 4: 8-15.

Doane, J. F. & R. K. Chapman, 1964. The relation of the cabbage maggot, *Hylemyia brassicae*, to decay in some cruciferous crops. *Ent. exp. appl.* 7: 1-8.

Graham, D. C., 1963. Serological diagnosis of potato blackleg and tuber soft rot. *Pl. Path.* 12: 142-144.

Graham, D. C., 1972. Identification of soft rot coliform bacteria. *Proc. 3rd int. Conf. Pl. pathogenic Bact.* (Wageningen, 1971). Pudoc, Wageningen, pp. 273-279.

Graham, D. C. & W. J. Dowson, 1960. The coliform bacteria associated with blackleg and other soft rots. I. Their pathogenicity in relation to temperature. *Ann. appl. Biol.* 48: 51-57.

Graham, D. C. & J. L. Hardie, 1971. Prospects for the control of potato blackleg disease by the use of stem cuttings. *Proc. 6th Br. Insectic. Fungic. Conf.* 1: 219-224.

Johnson. D. E., 1930. The relation of the cabbage maggot and other insects to the spread and development of soft rot of Cruciferae. *Phytopathology* 20: 857-872.

Lazar, I., 1972. Serological relationships between the 'amylovora', 'carotovora' and 'Herbicola' groups of the genus *Erwinia*. *Proc. 3rd int. Conf. Pl. pathogenic Bact.* (Wageningen, 1971). Pudoc, Wageningen, pp. 131-141.

Leach, J. G., 1926. The relation of the seed-corn maggot (*Phorbia fusciceps* Zett.) to the spread and development of potato blackleg. *Phytopathology* 16: 149-176.

Lempke, B. J., 1962. Insecten gevangen op het lichtschip 'Noord Hinder'. *Ent. Ber. Ned. ent. Ver.* 22: 100-111.

Molina, J., M. D. Harrison & J. W. Brewer, 1974. Transmission of *Erwinia carotovora* var. *atroseptica* by *Drosophila melanogaster*. I. Acquisition and transmission of the bacterium. *Am. Potato J.* 51: 245-250.

Ogilvie, L., B. O. Mulligan & P. W. Brian, 1935. Progress report on vegetable diseases. *A. Rep. Agric. hort. Res. Stn, Univ. Bristol.* 1934: 175-190.

Perombelon, M. C. M., 1973. Sites of contamination and numbers of *Erwinia carotovora* present in stored seed potato stocks in Scotland. *Ann. appl. Biol.* 74: 59-65.

Tamimi, K. M. & W. M. Banfield, 1969. Transmission of bacterial soft rot by fruit flies. *Phytopathology* 59: 403 (abstr.).

Yates, W. W. & A. W. Lindquist, 1952. Further studies on dispersion of flies tagged with radioactive phosphoric acid. *J. econ. Ent.* 45: 547-548.

Yerrington, A. P. & R. M. Warner, 1961. Flight distances of *Drosophila* determined by radioactive phosphorus. *J. econ. Ent.* 54: 425-428.

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Re-infection by *Erwinia carotovora* (Jones) Bergey et al. in Potato Stocks derived from Stem Cuttings[1)]

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ABSTRACT

Propagation of potato stocks by the stem cutting method has produced crops almost entirely free from infection with the soft rot coliform bacteria, *Erwinia carotovora* (Jones) Bergey et al var. *atroseptica* (Hellmers et Dowson) Dye and *E. carotovora* var. *carotovora* Dye. However, low levels of re-infection have occurred and evidence indicates that a source of the organisms was infected insects. Air-borne spread of bacterial aerosols generated by raindrop impaction on infected stems might also result in healthy stocks becoming re-infected.

Long experience has shown that potato blackleg and tuber soft rot (caused by soft rot coliform bacteria, *Erwinia carotovora* (Jones) Bergey et al. var. *atroseptica* (Hellmers et Dowson) Dye and *E. carotovora* var. *carotovora* Dye or var. *atroseptica* respectively) cannot be adequately controlled by agronomic methods, chemical treatments, or conventional certification procedures. By the mid 1960's, it became clear that soft rot coliforms were essentially tuber-borne. It was realized that, in common with certain important fungal diseases, these bacterial infections could be eliminated by raising stocks from cuttings, detached from stems before organisms spread into them. In 1967, the Department of Agriculture and Fisheries for Scotland (DAFS) initiated a project to produce seed from cuttings on a commercial scale, the eventual aim being to replace all commercial stocks with material derived from stem cuttings — now referred to as VTSC (i. e. virus-tested stem cutting) stocks. The process of replacement is still continuing.

To avoid re-infection as far as possible, DAFS raises the nuclear VTSC crops on a farm in an upland area where no commercial stocks are grown and under strict hygienic conditions. From 1967 to 1970, about 260,000 plants were grown on this farm and only 1, in the year 1969, was found infected with a soft rot coliform (*E. carotovora* var. *atroseptica*), but the source of infection was never traced. In 1971, 21 soft rot stem lesions on first year stem cuttings were found to be caused by *E. carotovora* var. *carotovora*. All these infections occurred on damaged areas or exposed leaf traces, and insects in the crop were found to be contaminated with the same organism. It was believed that the insects were spreading infection within the crop, but whether the insects introduced the bacteria into the crop cou'd not be determined. In July 1973 and 1974, dipterans (mainly species of *Leptocera*), found associated with a dump of decomposing vegetable matter nearby the farm, were contaminated with *E. carotovora* var. *carotovora*. Later in the season, infections by this organism were found on aerial parts of a few plants in the VTSC crop. The serotypes found associated with the dipterans

1) Summary of a paper presented a the EPPO Conference on Bacterial and Fungal Diseases of Potatoes, Brussels, 14-16 October, 1975.

were often the same as those isolated from the infected plants, and this, together with insect transmission experiments, provided compelling evidence that the sources of the organisms were the insects.

In no case was *E. carotovora* var. *atroseptica* isolated from insects, yet since 1971 a few plants infected with this organism have also been found each year on the farm. The source of the organisms remains unknown, although the possibility of insects travelling long distances cannot be ruled out. However, a consideration of other means of transport over a distance lead to the view that raindrop impaction on infected stems might generate bacterial aerosols which could then become wind-borne. In 1973, using a specially designed apparatus, it was demonstrated that raindrops could easily cause aerosol formation, and fractionation of the aerosols showed them probably to consist mainly of free bacterial cells. As many as 2.5×10^4 viable propagules could be released from 1 g of infected stem tissue. In experiments using Casella and Andersen airborne bacteria samplers, soft rot coliforms have been caught in the field downwind of potato crops during rain storms or when overhead irrigation sprinklers were in use. These observations add a new dimension to the problem of understanding the epidemiology of soft rot coliform infections. We do not know how significant airborne spread is in practice, but it should be possible, using these data, to eventually propose effective separation distances between ordinary commercial stocks and stem cutting stocks to avoid re-contamination.

In conclusion, it should be stressed that, despite the recurrence of infection with soft rot coliforms on stem cuttings, one should not lose sight of the fact that it has proved possible to produce stocks free from infection. We, in DAFS, are convinced that eventually we should be able to reduce the general level of infection in commercial crops to negligible proportions. A deeper understanding of the significance of insect and airborne transmission of soft rot coliform bacteria will help achieve that goal.

This is a summary of work which is continuing at Edinburgh ; more details can be found in :

— GRAHAM, D.C. & J.L. HARDIE (1971). Prospects for control of potato blackleg disease by the use of stem cuttings. *Proc. 6th Br. Insect. Fungic. Conf.* **1** : 219-224.

— GRAHAM, D.C. & M.D. HARRISON (1975). Potential spread of *Erwinia spp.* in aerosols. *Phytopathology* **65** : 739-741.

— GRAHAM, D.C., C.E. QUINN & M.D. HARRISON (1976). Recurrence of soft rot coliform bacterial infections in potato stem cuttings : an epidemiological study on the central nuclear stock production farm in Scotland. *Potato Res.* **19** : 3-20.

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RESUME

Réinfection par *Erwinia carotovora* (Jones) Bergey et al. de pommes de terre de base dérivées de bouturage par prélèvement sur tige

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La multiplication de semences de pommes de terre de base issues de bouturage par prélèvement sur tige a permis d'assurer une production pratiquement indemne des bactéries *Erwinia carotovora* (Jones) Bergey et al. var. *atroseptica* (Hellmers et Dowson) Dye et *E. carotovora* var. *carotovora* Dye. Dans certains cas, cependant, de faibles taux d'infection ont été enregistrés et, selon toute évidence, il faut en attribuer l'origine aux insectes. De plus, il est possible que les gouttelettes de pluie contaminées, transmises par le vent, jouent un rôle dans la dissémination des agents pathogènes.

Une meilleure compréhension de la part respective qu'il convient d'attribuer aux insectes et aux gouttelettes de pluie dans la diffusion de la maladie est encore nécessaire.

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Effect of fumigation of potato seed tubers with 2-aminobutane on plant growth and yield

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Summary

From 1968 to 1973, 13 field experiments were carried out to discover any effects of fumigation of seed tubers with 2-aminobutane (used for tuber disease control) on subsequent plant growth and yield. Nine experiments were done with tubers treated within 1 month of harvest, the remaining 4 with tubers stored for several months before treatment.

In general, fumigation did not have any substantial effect on the growth pattern or the yield of the crop obtained from treated seed, although in most cases it was probable that there were small increases in the number of sprouting eyes, percentage emergence, stem number and in weight and number of seed tubers at harvest. However, these effects rarely reached levels which gave a statistically significant increase over crops grown from untreated material.

Introduction

Graham et al. (1973a, b) showed that fumigation of tubers with gaseous 2-aminobutane gave very good control of the diseases gangrene (*Phoma exigua* var. *foveata*) and skin spot (*Oospora pustulans*) and controlled silver scurf (*Helminthosporium solani*) to some extent. Later Graham et al (1975) also showed that fumigation of visually healthy tubers prevented development of gangrene to a variable but often substantial extent in stocks that had been stored for several months before treatment.

This paper discusses the effects of 2-aminobutane fumigation on subsequent plant growth and yield from tubers treated up to one month after lifting or after 14 to 21 weeks of storage.

Materials and methods

The methods of fumigation employed were those described by Graham et al. (1973a). The 13 commercial stocks used for the experiments were handled and stored in the way briefly outlined by Graham et al. (1973a) and Graham et al. (1975); further details of treatments for the 9 stocks fumigated up to one month after lifting are summarized in Table 1, and for the 4 stocks fumigated after a period of storage in Table 2.

Table 1. Method of treatment of stocks up to one month after lifting.

Stock No[1]	Cultivar[2]	Date of harvest before treatment[3]	Method of grading[4]	Method of storage for fumigation[5]	Dosage*[6] (mg/kg)	Number of days elapsed between harvest and treatment[7]	Method of storage after treatment[8]	Experimental design in field[9]	Date of harvest of crop[10]
1	King Edward 1	17.10.68	not graded[11]	bulk[12]	200	1	bulk, then trays[13]	randomized block: 6 replicates[14]	30. 9.69
2	Majestic 1	22.10.68	do	do	200	1	do	do	30. 9.69
3	Majestic 2	23.10.68	do	trays[15]	280	2	trays	do	30. 9.69
					280	7			
					280	14			
4	Redskin 1	7.10.69	do	bulk	200	1	bulk, then trays	do	6.11.70
					200	2			
					200	14			
5	King Edward 2	15.10.69	do	do	200	1	do	do	6.11.70
					200	14			
6	Redskin 2	13.10.70	riddle[16]	do	50	1	do	latin square:[17] 5×5	1.11.71
					200	2			
					200	14			
					200	30			
7	King Edward 3	7.10.70	do	do	50	1	do	do	1.11.71
					200	2			
					200	13			
					200	27			
8	Pentland Crown 1	5.10.71	spool[18]	do	50	2	do	factorial: 5 replicates[19]	24.10.72
					200	2			
					50	29			
					200	29			
9	King Edward 4	1.10.71	do	do	50	1	do	do	25.10.72
					200	1			
					50	29			
					200	29			

* For controls, tubers from each stock were left untreated – *Als Vergleich blieben Knollen von jeder Partie unbehandelt – Comme témoins, des tubercules de chaque lot furent conservés non traités.*

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[1] *Nummer der Partie – Numéro du lot;* [2] *Sorte – Variété;* [3] *Datum der Ernte vor der Behandlung – Date de récolte avant le traitement;* [4] *Art der Sortierung – Méthode de triage;* [5] *Lagerungsart vor der Begasung – Méthode d'entreposage pour la fumigation;* [6] *Dosis – Dose;* [7] *Zahl der zwischen Ernte und Behandlung vergangenen Tage – Nombre de jours écoulés entre la récolte et le traitement;* [8] *Lagerungsart nach der Behandlung – Méthode d'entreposage après le traitement;* [9] *Anlage des Feldversuches – Dispositif expérimental en champ;* [10] *Datum der Ernte – Date de récolte de la descendance;* [11] *Nicht sortiert Non trié;* [12] *Haufen – En vrac;* [13] *Haufen, dann Kisten – En vrac, puis en clayettes;* [14] *Randomisierte Blocks: 6 Wiederholungen – Bloc randomisé: 6 répétitions;* [15] *Kisten – En clayettes;* [16] *Sieb – Trieur à grilles;* [17] *Lateinisches Quadrat – Carré latin;* [18] *Walzen – Trieur à 'bobines' rotatives;* [19] *Faktoriell: 5 Wiederholungen – Factoriel: 5 répétitions.*

Tabelle 1. Methode der Behandlung der Partien bis zu einem Monat nach der Ernte.
Tableau 1. Méthode de traitement des lots désinfectés moins d'un mois après l'arrachage.

The following growth and yield characters were measured:
- number of eyes sprouted by mid-April on 50 treated tubers
- number of sprouts over 1 cm long by mid-April on 50 treated tuber
- percentage emergence of crop by end of June
- number of stems per plant in late July
- plant height in late July
- total yield of tubers by weight and by number
- yield of seed and ware (table) tubers separately by weight and by number.

All the experimental material was planted in the last week in April each year. In the earlier experiments (1969–71), the weight of tubers planted in each plot was not standardized but variation in weight was reduced by discarding the smaller and larger tubers. After 1971 plots were planted with the same weight of tubers.

The experimental designs of the trials are given in Tables 1 and 2. Randomized blocks were replicated 6 times, and the latin squares 5 × 5. The remaining trials were factorials which had either 5 or 6 replicates. Plots in each lay-out consisted of 4 rows of tubers with 30 tubers per row. For determining emergence, plants were counted in all 4 rows. Counts of the number of stems per plant and height measurements were done on 10 plants in the plot – 2 each from the outer and 3 each from the inner rows. Plant height was determined by measuring the distance between the top of the drill and the top of the tallest stem. For yield determination, the crop from only the two centre rows was lifted, the other rows being used as guards.

The plots were cultivated in a conventional manner and protective sprays of mancozeb against potato blight (*Phytophthora infestans*) applied as necessary. In the years 1969–71, haulm had to be destroyed in mid-September to stop further spread of blight but in 1972 and 1973 crops were allowed to come to full maturity before harvesting. The tubers were graded into 3 categories using 57 mm × 32 mm hand riddles and the weights and numbers determined.

Most statistical calculations were made using the computers of the Edinburgh Regional Computer Centre. In all cases, any differences between treatments were accepted as significant at $P < 0.05$.

Table 2. Method of treatment of stocks after a period of storage.

Stock No[1]	Cultivar[2]	Period of storage before treatment[3]	Method of grading[4]	Method of storage for fumigation[5]	Dosage[6]* (mg/kg)	Number of days elapsed between grading and treatment[7]	Method of storage after treatment[8]	Experimental design in field[9]	Date of harvest of crop[10]
10	Pentland Crown 2	20 weeks[11]	riddle[16]	trays[15]	200	1	trays	randomised block: 6 replicates[14]	1.11.71
					200	8			
11	Maris Peer 1	21 weeks	spool[18]	do	200	2	do	factorial[16]: 5 replicates	24.10.72
			riddle	do	200	2	do		
					200	9			
12	Pentland Dell 1	20 weeks	spool	do	200	1	do	factorial[19]: 6 replicates	30.10.73
					200	8			
			riddle	do	200	2	do		
					200	9			
13	Pentland Dell 2	14 weeks	spool	do	200	1	do	do	30.10.73
					200	8			
			riddle	do	200	0	do		
					200	7			

* For controls, each stock had tubers left unfumigated from riddled and spool graded treatments – *Als Vergleich blieben von jeder Partie Knollen unbehandelt, die über Siebe und Walzen sortiert worden waren – Comme témoins, des tubercules de chaque lot furent conservés non traités après le triage.*

[1, 2, 4, 5, 6, 8, 9, 10, 14, 15, 16, 18, 19] *Siehe Tabelle 1 – Voir tableau 1;* [3] *Zeitraum der Lagerung vor der Behandlung – Durée d'entreposage avant le traitement;* [7] *Zahl der zwischen Sortierung und Behandlung vergangenen Tage – Nombre de jours écoulés entre le triage et le traitement;* [11] *Wochen – Semaines.*

Tabelle 2. Art der Behandlung der Partien nach einer Periode der Lagerung.
Tableau 2. Méthode de traitement des lots désinfectés après une période d'entreposage.

Results

Analyses generated a great deal of conventional statistical data; it was not considered necessary to reproduce these in detail, but by way of example data from experiments 9 and 11 are presented in Tables 3 and 4.

The development of eyes was not affected by fumigation in experiments 2, 5, 11 and 12. In experiments 3, 10 and 13 results were not significant except for one treatment in each trial where either more eyes sprouted or more sprouts exceeded 1 cm in length. In experiments 1, 4, 6, 7, 8 and 9 significant increases in the number of growing eyes were observed as a result of fumigation.

As regards other growth and yield characters, 4 of the 13 trials (3, 4, 9 and 11) gave results which showed that there were no significant differences in any aspect of growth or yield of the subsequent crop which could be attributed to the use of 2-aminobutane.

Of the remaining 9 experiments, 7 showed no differences between fumigated and untreated seed in respect of percentage emergence, whereas in trials 1 and 5 there was a small significant increase in emergence from treated tubers. The number of stems reaching maturity was unaffected in 5 experiments (1, 2, 7, 8 and 13) and significantly increased in 4 (5, 6, 10 and 12). There was no change in the height of stems. The total weight of tubers in the 9 experiments was not affected, but as regards the number of tubers, there were 2 trials (1 and 6) in which a significant increase was found. In 5 trials (2, 5, 6, 7 and 13) the weight of ware and in 2 (5 and 6) the number of ware tubers were significantly decreased by fumigation. Despite the constant total yield, the compensating weight increase in the seed fraction was statistically significant in only one case (experiment 6); in this case the number of seed tubers also rose significantly. In no case was there a significant increase in the weight and number of tubers in the ware fraction, or a significant decrease in the weight and number of the seed fraction.

Conclusion

The experiments indicate that the use of 2-aminobutane as a fumigant for seed potatoes does not cause any disadvantageous effects on growth pattern or yield of the crop obtained from treated seed. There may be a small effect in terms of the number of sprouting eyes, the percentage emergence, stem number and in weight and number of seed tubers at harvest, for the results show a generally consistent increase in all these features although acceptable levels of significance were reached in only a few cases. However, the purpose of fumigation is to control tuber diseases in storage and any benefits in growth and yield should be regarded as a bonus for the grower.

Another noteworthy aspect of the experiments is that the untreated seed used had been subjected to rigorous selection for presence of disease and only tubers visually disease-free were planted. This would not generally happen in commerce and could have influenced the results so that significant differences between treated and untreated material were not usually achieved. Commercial companies have informed us that,

Table 3. Results of experiment 9 – tubers of cv King Edward treated at 1 and 29 days after harvest.

Growth and yield characters[2]	Treatment (see Table 1)[1]						
	nil[3]	50 mg/kg day[4] 1	200 mg/kg day 1	50 mg/kg day 29	200 mg/kg day 29	average[5] SE[+]	CV[++] (%)
Average number of eyes per tuber[6]	3.16	3.98**	3.90**	4.30**	4.26**	1.20	30.7
Average number of eyes per tuber with sprouts over 1 cm long[7]	1.74	2.14	2.02	2.12	2.16	0.99	48.7
Emergence[8] (%)	98.33	99.50	99.17	98.83	99.00	0.452	1.02
Average number of stems per plant[9]	3.54	3.42	3.94	3.56	3.88	0.196	11.95
Stem height[10] (cm)	76.10	76.64	74.38	77.82	77.18	1.09	3.20
Yield per plot[11] (kg)	80.04	81.35	78.25	80.31	76.70	2.77	7.81
Ware weight per plot[12] (kg)	49.35	46.72	40.73	45.86	42.57	2.85	14.16
Ware number per plot[13]	301.60	311.40	271.80	300.20	289.40	17.47	13.25
Seed weight per plot[14] (kg)	29.08	33.00	35.68	32.91	32.52	1.06	7.24
Seed number per plot[15]	474.80	547.00	590.00	546.20	531.20	18.43	7.69

[+] SE = Standard Error – *Standardabweichung – Erreur standard.*
[++] CV = Coefficient of Variation – *Variationskoeffizient – Coefficient de variation.*
** $P < 0.01$; all other differences between tubers fumigated and not fumigated were not significant at $P < 0.05$ – *$P < 0,01$; alle anderen Differenzen zwischen begasten und unbegasten Knollen sind nicht signifikant bei $P < 0,05$ – $P < 0,01$; toutes les autres différences entre des tubercules désinfectés et non désinfectés ne sont pas significatives à $P < 0,05$ – $P < 0,01$.*

[1] *Behandlung siehe Tabelle 1 – Traitement voir tableau 1;* [2] *Wuchsund Ertragseigenschaften – Caractères concernant la croissance et le rendement;* [3] *Unbehandelt – Témoin non traité;* [4] *Tag – Jour;* [5] *Mittlere – Moyenne;* [6] *Mittelwert der Zahl der Augen pro Knolle – Nombre moyen d'yeux porteurs de germes par tubercule;* [7] *Mittelwert der Zahl der Augen pro Knolle mit über 1 cm langen Keimen – Nombre moyen d'yeux portant des germes de plus d'un cm de long par tubercule;* [8] *Auflaufrate – Plantes levées;* [9] *Mittelwert der Stengelzahl pro Pflanze – Nombre moyen de tiges par plante;* [10] *Stengelhöhe – Hauteur des tiges;* [11] *Ertrag pro Parzelle – Rendement par parcelle;* [12] *Gewicht der Speisekartoffeln pro Parzelle – Poids de pommes de terre de calibre de consommation ('dessus de plant') par parcelle;* [13] *Zahl der Speisekartoffeln pro Parzelle – Nombre de pommes de terre de calibre de consommation ('dessus de plant') par parcelle;* [14] *Gewicht der Saatkartoffeln pro Parzelle – Poids de pommes de terre de calibre de semence par parcelle;* [15] *Zahl der Saatkartoffeln pro Parzelle – Nombre de pommes de terre de calibre de semence par parcelle*

Tabelle 3. Ergebnisse von Versuch 9 – Knollen der Sorte King Edward 1 bzw. 29 Tage nach der Ernte behandelt.
Tableau 3. Rèsultats de l'essai no. 9 – tubercules de la variété King Edward désinfectés 1 jour et 29 jours après la récolte.

Table 4. Results from experiment 11 – tubers of cv Maris Peer treated after 21 weeks of storage and at 2 and 9 days after grading.*

Growth and yield characters[2]	Treatment (see Table 2)[1]					Average[5] SE+	CV++ (%)
	spool-graded[16]		riddle-graded[17]				
	nil[3]	200 mg/kg day[4] 2	nil	200 mg/kg day 2	200 mg/kg day 9		
Average number of eyes per tuber[6]	4.78	4.62	4.80	4.72	4.48	1.15	24.5
Average number of eyes per tuber with sprouts over 1 cm long[7]	4.60	4.46	4.44	4.22	4.36	1.53	34.6
Emergence[8] (%)	96.0	97.67	98.33	99.33	97.33	0.899	2.06
Average number of stems per plant[9]	3.20	3.56	3.44	3.30	3.40	0.192	12.73
Stem height[10] (cm)	52.08	54.50	53.48	53.52	53.18	0.924	3.87
Yield per plot[11] (kg)	63.48	65.61	67.40	64.73	68.49	1.62	5.49
Ware weight per plot[12] (kg)	24.56	25.58	27.58	25.02	30.64	2.05	17.21
Ware number per plot[13]	176.20	185.20	192.00	187.40	219.20	13.31	15.49
Seed weight per plot[14] (kg)	38.06	39.08	38.96	38.94	36.92	1.29	7.51
Seed number per plot[15]	612.00	632.40	620.00	626.20	618.20	31.18	11.21

+, ++ See Table 3 – Siehe tabelle 3 – Voir tableau 3

* All differences between tubers fumigated and not fumigated were not significant at $P < 0.05$ – *Alle Differenzen zwischen begasten und unbegasten Knollen sind nicht signifikant bei $P < 0{,}05$ – Toutes les différences entre les tubercules désinfectés et non désinfectés ne sont pas significatives au niveau $P < 0{,}05$.*

[1–15] *Siehe Tabelle 3 – Voir tableau 3;* [16] *Sortiert mit einem Walzensortiergerät – Trieur à 'bobines' rotatives;* [17] *Sortiert mit einem Schwingsieb – Trieur à grilles*

Tabelle 4. Ergebnisse von Versuch 11 – Behandlung der Knollen der Sorte Maris Peer nach 21 Wochen Lagerung und 2 und 9 Tage nach der Sortierung.*

Tableau 4. Rèsultats de l'essai no. 11 – tubercules de la variété Maris Peer désinfectés après 21 semaines d'entreposage, 2 jours et 9 jours après triage.*

in their experience, treated seed has, in some instances at least, yielded substantially larger crops than untreated material.

Acknowledgment

We would like to thank staff of the Computer Services Unit of the East of Scotland College of Agriculture for help with the statistical analyses.

Zusammenfassung

Einfluss der Begasung von Saatkartoffeln mit 2-Aminobutan auf das Pflanzenwachstum und den Ertrag

Während der Jahre 1968–1973 wurden 13 Feldversuche durchgeführt, um den Einfluss der Begasung von Saatkartoffeln mit 2-Aminobutan (das zur Vermeidung von Knollenkrankheiten verwendet wird) auf das Pflanzenwachstum und den Ertrag im nächsten Jahr zu untersuchen. Folgende Wachstums- und Ertragskriterien wurden bestimmt:

- Mitte April die Zahl der gekeimten Augen von 50 behandelten Knollen
- Mitte April die Zahl der über 1 cm langen Keime von 50 behandelten Knollen
- Ende Juni die Auflaufrate des Saatgutes in Prozent
- Ende Juli die Stengelzahl pro Pflanze
- Ende Juli die Höhe der Pflanzen
- Gesamtertrag der Knollen durch Feststellung des Gewichtes und der Zahl
- Ertrag an Saat- bzw. Speisekartoffeln durch Feststellung des Gewichtes und der Knollenzahl.

Neun Versuche wurden mit Knollen durchgeführt, die innerhalb eines Monats nach der Ernte behandelt wurden (Tabelle 1), die restlichen 4 mit Knollen, die vor der Behandlung mehrere Monate gelagert wurden (Tabelle 2). Die Ergebnisse sind für den Versuch 9, Behandlung der Knollen bis zu einem Monat nach der Ernte (Tabelle 3) und den Versuch 11, Behandlung nach 21 Wochen Lagerung (Tabelle 4) dargestellt.

Im allgemeinen hatte die Begasung der Saatkartoffeln keinen signifikanten Effekt auf das Wuchsbild oder den Ertrag, obgleich es in einer Reihe von Fällen so aussah, als ob sich ein geringer Einfluss ergeben hätte in Richtung auf eine Steigerung der Zahl gekeimter Augen, der Auflaufrate in Prozent, der Stengelzahl und des Gewichtes und der Zahl der Saatkartoffeln bei der Ernte. Diese Steigerungen liessen sich jedoch nur selten gegenüber unbehandeltem Saatgut statistisch sichern. Der Zweck der Begasung ist die Kontrolle von Krankheiten während der Lagerung und jeder Vorteil im Wachstum und im Ertrag sollte als ein Erfolg des Anbauers gesehen werden. Ebenso muss gesagt werden, dass unter Versuchsbedingungen das unbehandelte Saatgut einer rigorosen Selektion unterworfen ist und so nur augenscheinlich gesundes Saatgut gepflanzt wird. Das geschieht normalerweise nicht im kommerziellen Anbau und könnte die Ergebnisse so beeinflusst haben, dass signifikante Unterschiede zwischen behandeltem und unbehandeltem Saatgut im allgemeinen nicht erreicht wurden.

Résumé

Effet du traitement des tubercules de semence par fumigation au 2 aminobutane sur la croissance des plantes et leur rendement

Au cours des années 1968–73, 13 essais de plein champ ont été réalisés afin de connaître les effets de la fumigation des tubercules de semence au 2 aminobutane (utilisé pour combattre les maladies du tubercule) sur la croissance des plantes qui en sont issues et leur rendement. Les caractères suivants, concernant la croissance et le rendement, ont été mesurés:

- nombre d'yeux porteurs de germes à la mi-avril (sur 50 tubercules)
- nombre de germes de plus d'un cm de long à la mi-avril (sur 50 tubercules)
- pourcentage de plantes levées à la fin du mois de juin
- nombre de tiges par plante fin juillet
- hauteur des plantes fin juillet
- rendement total en poids et en nombre de tubercules
- rendement respectif en calibres de semences et en calibres de consommation en poids et en nombre

9 essais ont été réalisés avec des tubercules traités moins d'un mois après la récolte (tableau 1), les quatre autres avec des tubercules traités plusieurs mois après le stockage (tableau 2).

A titre d'exemple, les résultats sont donnés pour l'essai no 9, lot traité moins d'un mois après l'arrachage (tableau 3) et l'essai no 11, lot traité après 21 semaines d'entreposage (tableau 4).

En général, la fumigation n'a eu aucun effet significatif sur la croissance et le rendement de la récolte obtenue à partir de semences traitées, bien que dans un certain nombre de cas il soit probable qu'il y ait eu un léger effet en faveur d'une augmentation du nombre d'yeux porteurs de germes, du pourcentage de plantes levées, du nombre de tiges, du poids et du nombre de tubercules de calibres de semence récoltés.

Cependant ces effets ont rarement atteint des niveaux donnant une amélioration statistiquement significative par rapport aux récoltes obtenues à partir de matériel non traité.

Quoiqu'il en soit, le but de la fumigation étant avant tout de combattre les maladies durant la conservation, tout avantage au niveau de la croissance et du rendement ne devrait être considéré que comme une bonification pour le producteur. Il conviendrait aussi de noter que, dans les conditions de cette expérimentation, la semence non traitée a fait l'objet d'un choix rigoureux de telle sorte que, seule, une semence apparemment saine a été plantée. Ceci ne se produirait pas pratiquement et pourrait avoir influencé les résultats en faveur de la semence non traitée.

References

Graham, D. C., G. A. Hamilton, C. E. Quinn & A. D. Ruthven, 1973a. Use of 2-aminobutane as a fumigant for control of gangrene, skin spot and silver scurf diseases of potato tubers. *Potato Res.* 16: 109–25.

Graham, D. C., G. A. Hamilton, M. J. Nash & J. H. Lennard, 1973b. Fumigation of bulk-stored potatoes with 2-aminobutane for control of gangrene, skin spot and silver scurf diseases. *Potato Res.* 16: 234–43.

Graham, D. C., G. A. Hamilton, C. E. Quinn & A. D. Ruthven, 1975. Control of potato gangrene by fumigation of tubers with 2-aminobutane after periods of storage. *Potato Res.* 18: 410–5.

Potato Res. 20 (1977): 37–52

Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to re-contamination of pathogen-free potato stocks

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Zusammenfassung, Résumé p. 50

Keywords: waste, potato, insects, soft rot coliform bacteria, *Erwinia carotovora* var. *carotovora*, *Erwinia carotovora* var. *atroseptica*

Summary

Insects belonging to 12 genera in the Order Diptera, found at two large waste potato dumps in Scotland, were contaminated with soft rot coliform bacteria. In 1973, 5.7% and 3.2%, and in 1974, 4.8% and 4.1% of the insects caught at each site yielded these organisms. The bacteria were identified mainly as *Erwinia carotovora* var. *carotovora*, though some isolates were *Erwinia carotovora* var. *atroseptica*. Isolations from potato dump waste showed that at the time when fly activity was greatest the majority of soft rot coliforms in the waste were *E. carotovora* var. *carotovora*, despite the fact that tubers originally forming the dumps were almost certainly infected mostly with *E. carotovora* var. *atroseptica*. Contaminated insects could readily transmit soft rot coliforms to damaged areas on the aerial parts of potato plants. It is suggested that potato stocks freed from these organisms by the stem cutting procedure could become re-contaminated by insects that disperse from potato dumps.

Introduction

After the development of blackleg-free potato stocks in Scotland by the stem cutting method, many more critical observations have become possible on the epidemiology of potato blackleg caused by *Erwinia carotovora* var. *atroseptica* (subsequently referred to as *E. atroseptica*), and of tuber soft rot, caused both by *E. carotovora* var. *carotovora* (subsequently referred to as *E. carotovora*) and by *E. atroseptica*. Previously, the very high incidence of latent infection with these organisms prevented clarification of how they might spread from crop to crop. Cases of infection have been investigated in stocks derived from stem cuttings since their release into commerce in 1970, with the aim of finding how to avoid or prevent re-contamination. As expected, machinery such as sprayers and graders was shown to be often contaminated. However, it soon became clear that it would be much harder to find the origin of the *Erwinia* bacteria

which established low levels of re-infection in some crops. Notably the majority of the infections occurred on the aerial parts of the plants through petiole scars, broken petioles or crushed or otherwise injured stems (Graham & Hardie, 1971). Such observations suggested aerial dissemination in unknown ways from unknown sources. Studies of infections on plants grown at the central nuclear stock production farm in Scotland over the years 1971 to 1974 indicated that an important likely source was dipterous insects associated with a dump of waste vegetable matter nearby. It was shown that the organism isolated from the insects was *E. carotovora* and that the several serotypes of this bacterium obtained from the insects were subsequently found associated with infections on plant haulm (Graham et al., 1976).

The need for further information about the potential importance of insects as sources of re-contamination in the general environment prompted the following studies of organisms associated with insects found at potato dumps. Bacteria in the dump debris were also isolated and identified. Dumps are very widely distributed throughout the potato growing areas of Scotland and could be the place of origin of contaminated insects. Boyd (1974) has already drawn attention to dumps as the most important foci from which late blight (*Phytophthora infestans*) infections become established in the field.

Methods

Collection and bacteriological investigation of insects from dumps

Two large potato dumps, one at Perth, another at Burrelton, almost 30 km apart, were selected for study. Both lay in an important seed potato growing area. Each was visited 15 times at varying intervals beginning 7 June and ending on 24 October 1973, and 11 times from 12 June until 11 November 1974. During each visit, which usually took place in the late morning or early afternoon, a random collection of insects was taken by sweeping across the dumps with an insect net, or by catching insects individually in McCartney bottles, although the latter method was used only occasionally. Individual insects or groups of insects were transferred from the net to McCartney bottles, and returned to the laboratory for identification and testing for soft rot coliform bacteria. Numbers varied somewhat in relation to such factors as weather conditions and time of year. However, in 1973 usually 30–35, and in 1974 70–80, insects were obtained from each site at each visit.

The methods used for isolating, purifying and identifying the bacteria associated with the insects were the same as those described by Graham et al. (1976). Representative samples of insects were identified, usually to species.

Bacteria in debris from dumps

As the organisms found associated with the insects very probably originated from the dump debris, soft rot coliform bacteria in debris were isolated and identified. Samples were taken from numerous places at the sites in 1975. At each place, 3 sub-samples, each weighing about 170 g, were collected at different depths (0–9 cm, 10–15 cm and

30–38 cm) to determine if bacterial populations varied at different levels. The samples were placed in polythene bags and well mixed before testing the following day. About 0.5–1.0 g of obvious tuber debris (when present) and about 5.0 g of decomposed tuber and soil mixture were suspended in 20 ml sterile water and allowed to settle, after which a 2 mm diameter loopful of the supernatant was plated on each of two Stewart's pectate gel plates. Pectolytic organisms were purified and identified as described by Graham et al. (1976).

Transmission of soft rot coliform bacteria to potato plants by insects collected at a dump
An experiment was made to test the ability of insects collected at Perth in August 1973 to transmit soft rot coliforms to the aerial parts of potato plants. Fifteen plants of pathogen-free potatoes of cv. Pentland Javelin, were grown in a greenhouse. When they were about 25 cm high, the number of stems per pot was reduced to 5, and plants divided into 3 groups of 5 plants each. One group received no treatment; in the 2 remaining groups single stems on each plant were injured in the following 5 ways: by breaking off the stem 20 cm above soil level; by breaking 2 petioles at the point of attachment to the stem; by breaking 2 petioles half way between the stem and the terminal leaflet; by gouging out tissue from the side of the stem in 2 places; and by crushing 2 areas of the stem with forceps. Plants in all groups were covered with plastic bags and shaded. A random collection of 50 flies was released into each of the 5 plastic bags covering the plants used to test insect transmission, the other 2 groups of plants (1 injured, 1 uninjured) acting as controls. The collections included members of the genera *Leptocera*, *Drosophila* and *Parascaptomyza*. Tests for soft rot coliform bacteria on fly samples collected at Perth on the same date showed that 11.4% were contaminated with *E. carotovora*.

Flies were confined within the bags for 3 days, then released, and bags removed from all the plants. The stems and petioles were periodically examined to observe any soft rot symptoms, and small pieces of injured tissue were removed at the end of a further 4 days, crushed in sterile water and the resulting suspension plated on Stewart's pectate gel. Pectolytic colonies were removed for purification and identification as before.

Results

Insects and bacteria found associated in 1973
The identity and numbers of isolates of *Erwinia* sp. obtained from the insects are summarized by location and by date in Table 1; and *Erwinia* sp. contamination of insects collected at Perth and Burrelton are given in Tables 2 and 3, respectively.

No insects contaminated with soft rot coliforms were found in the first 2 collections, but after 15 June (excepting 3 July) these organisms were consistently isolated from dipterans at both sites. Combining data from both locations, the proportion of contaminated insects generally increased into the summer and autumn, with as many as 9.4% of the insects collected on 8 August and 24 October yielding the bacteria.

Table 1. Isolation of *Erwinia carotovora* var. *atroseptica* and *Erwinia carotovora* var. *carotovora* from insects by location and date, 1973.

Date[1]	Number of isolates of each variety[2]			
	Perth		Burrelton	
	var. *atroseptica*	var. *carotovora*	var. *atroseptica*	var. *carotovora*
7 June	0	0	0	0
15 June	0	0	0	0
22 June	0	1	1	0
27 June	0	1	0	0
3 July	0	0	0	0
13 July	0	2	0	1
23 July	1	0	1	0
30 July	1	4	0	1
8 August	0	3	0	3
21 August	0	2	0	1
28 August	0	3	0	1
10 September	0	4	0	2
24 September	0	3	0	1
10 October	0	2	0	0
24 October	0	2	0	1
Total	2	27	2	11

[1] *Datum – Date;* [2] *Anzahl Isolate von jeder Sorte – Nombre d'isolement pour chaque variété*

Tabelle 1. Isolation von *Erwinia carotovora* var. *atroseptica* und *Erwinia carotovora* var. *carotovora* von Insekten nach Ort und Datum, 1973.
Tableau 1. Isolement d'*Erwinia carotovora* var. *atroseptica* et *Erwinia carotovora* var. *carotovora* à partir d'insectes en fonction du lieu et de la date, 1973.

Somewhat unexpectedly, *E. atroseptica* was isolated only 4 times. This organism was obtained from both Perth and Burelton, but only early in the season, on or before 30 July. *E. carotovora* was isolated 38 times and consistently throughout the sampling period. The ratio *E. carotovora* to *E. atroseptica* was 9.5:1 when data from the 2 sites were combined; at Perth the ratio was 13.5:1 and at Burrelton 5.5:1. Six genera of Diptera were found contaminated, whereas 2 other Diptera and certain miscellaneous insects did not yield soft rot coliforms. Representative samples of insects were identified as: *Leptocera ferruginata*, *L. fontinalis*, *Scatopse notata*, *S. fuscipes*, *Sepsis violacea*, *Coenosia tricolor*, *Parascaptomyza pallida*, *Drosophila buscki*, *D. hydei* and *Azelia* sp. Where there was more than one species in a genus, similarities between them usually did not allow specific identifications to be made before the insects were destroyed in the process of isolating bacteria from them, so that in most cases it was impossible to relate the presence of soft rot coliform bacteria to particular species.

Essentially the same insect fauna was obtained from both locations, although *Azelia* sp. was caught only at Perth. Insect activity was greater at Perth than at

Table 2. Contamination by *Erwinia* sp. of insects collected from Perth, June-October 1973.

	Number collected[1]	Percentage of total insects collected[2]	Number contaminated with *Erwinia*[3]	Percentage contaminated with *Erwinia*[4]	Percentage of total number of *Erwinia* isolates[5]
Azelia sp.	34	6.9	2	5.9	7.1
Coenosia tricolor	25	5.1	2	8.0	7.1
Drosophila spp.	73	14.9	7	9.6	25.0
Leptocera spp.	184	37.4	6	3.3	21.4
Parascaptomyza pallida	67	13.6	10	14.9	35.7
Scatopse spp.	54	11.0	1	1.9	3.6
Sepsis spp.	34	6.9	0	0.0	0.0
Other Diptera[6]	12	2.4	0	0.0	0.0
Other insects[7]	9	1.8	0	0.0	0.0

[1] *Anzahl gesammelter Insekten – Nombre de capturés;* [2] *% totaler Insekten gesammelt – Pourcentage d'insectes totals capturés;* [3] *Anzahl kontaminiert mit Erwinia – Nombre de contaminés par Erwinia;* [4] *% kontaminiert mit Erwinia – Pourcentage de contaminés par Erwinia;* [5] *Gesamtzahl der Erwinia-Isolate in % – Poutcentage du nombre total d'isolements d'Erwinia;* [6] *Andere Zweiflügler – Autres diptères;* [7] *Andere Insekten – Autres insectes*

Tabelle 2. Kontamination von in Perth gesammelten Insekten mit *Erwinia* sp., Juni-Oktober 1973.
Tableau 2. Contamination par *Erwinia* sp. des insectes capturés à Perth, juin-octobre 1973.

Table 3. Contamination by *Erwinia* sp. of insects collected from Burrelton, June-October 1973.

	Number collected[1]	Percentage of total insects collected[2]	Number contaminated with *Erwinia*[3]	Percentage contaminated with *Erwinia*[4]	Percentage of total number of *Erwinia* isolates[5]
Coenosia tricolor	44	11.0	2	4.6	15.4
Drosophila spp.	2	0.4	1	50.0	7.7
Leptocera spp.	163	40.6	6	3.9	46.1
Parascaptomyza pallida	17	4.2	3	17.7	23.1
Scatopse spp.	109	27.2	1	0.9	7.7
Sepsis spp.	14	3.6	0	0.0	0.0
Other Diptera[6]	26	6.5	0	0.0	0.0
Other insects[7]	26	6.5	0	0.0	0.0

[1 – 7] *Siehe Tabelle 2 – Voir tableau 2*

Tabelle 3. Kontamination von in Burrelton gesammelten Insekten mit *Erwinia* sp., Juni-Oktober 1973.
Tableau 3. Contamination par *Erwinia* sp. des insectes capturés à Burrelton, juin-octobre 1973.

Burrelton, possibly due to the dumping of large quantities of waste potatoes at Perth early in 1973, whereas potatoes had not been deposited at Burrelton for about a year before this study began and there was no obvious rotting material. Combined data from Perth and Burrelton show that of 893 insects tested 41 (4.6%) were contaminated with soft rot coliforms. The fruit flies (*Drosophila* spp. and *Parascaptomyza pallida*) were the most frequently contaminated. While these insects represented less than 19% of the insects they yielded 51% of the *Erwinia* isolates. *Leptocera* spp. and *Scatopse* spp. represented 57.1% of the insects collected, but yielded only 34.1% of the isolates. The muscids *Coenosia* sp. and *Azelia* sp. amounted to 11.5% of the catch and yielded 14.6% of the isolates. *Sepsis* spp. and miscellaneous insects such as small moths, a few aphids, small beetles and some unidentified muscids were not contaminated.

During the course of the season, marked changes occurred in the composition of the insect population. Some species appeared in large numbers early and then declined (e.g. *Coenosia* sp.). Others, such as *Azelia* sp., did not appear until September. *Leptocera* spp. and *Scatopse* spp. were present throughout the collecting period, but maximum populations developed from mid-June to mid-July and then gradually declined. However these observations should be treated as generalizations only, because insect emergence and activity is affected by weather conditions and other factors, such as time of day (cf. Johnson, 1969, on *Drosophila*).

Insects and bacteria found associated in 1974

Results of the 1974 experiments are given in Tables 4, 5 and 6. The pattern of the catch and its association with *Erwinia* sp. was similar to that for 1973. Insects contaminated with soft rot coliforms were found at both sites in every sample except at Perth on 4 July and 24 September. When data from both sites were combined the proportion of contaminated insects generally increased from June through July. On 30 July 8.8% of the catch yielded soft rot coliform bacteria. In August and September the number of contaminated insects fell, but increased sharply in October and November to 13.8% and 14.7%, respectively.

E. atroseptica was isolated 18 times and was found both at Perth and Burrelton. These isolates were obtained either early in the season (as in 1973) or in the months of October and November. *E. carotovora* was isolated 69 times and was consistently obtained throughout the sampling period, as in 1973. The ratio of *E. carotovora* to *E. atroseptica* found at Perth and Burrelton was 3.8:1 when data from both sites were combined. At Perth, the ratio was 2.7:1 and at Burrelton 6.8:1.

Ten genera of the Order Diptera were found to be contaminated with soft rot coliforms. Of the large number of unidentified dipterans and other insects tested from both sites only 1.1% yielded these bacteria. Representative samples of the insects were identified as far as possible to species and the following were found in addition to those caught in 1973: *Leptocera vagans*, *L. humida*, *L. rufilabris*, *L. caenosa*, *L. mirabilis*, *L. moesta*, *Drosophila funebris*, *Themira minor*, *T. putris*, *Nemopoda nitidula*, *Nupedia infirma*, *Scatophaga squalida*, *S. stercoraria* and *Delia florilega*; other *Delia* spp. and *Azelia* sp. could not be specifically identified. The increased

Table 4. Isolation of *Erwinia carotovora* var. *atroseptica* and *Erwinia carotovora* var. *carotovora* from insects by location and date, 1974.

Date[1]	Number of isolates of each variety[2]			
	Perth		Burrelton	
	var. *atroseptica*	var. *carotovora*	var. *atroseptica*	var. *carotovora*
12 June	0	2	0	1
24 June	1	1	0	1
4 July	0	0	0	2
17 July	1	7	0	2
30 July	3	7	1	8
13 August	0	5	0	5
27 August	0	1	0	2
9 September	0	6	0	1
24 September	0	0	0	2
10 October	1	3	3	6
6 November	7	3	1	4
Total	13	35	5	34

[1–2] *Siehe Tabelle 1 – Voir tableau 1*

Tabelle 4. Isolation von *Erwinia carotovora* var. *atroseptica* und *Erwinia carotovora* var. *carotovora* von Insekten nach Ort und Datum, 1974.

Tableau 4. Isolement d'*Erwinia carotovora* var. *atroseptica* et *Erwinia carotovora* var. *carotovora* à partir d'insectes en fonction du lieu et de la date, 1974.

number of insects tested added greatly to the amount of taxonomic work and it was not always possible to have insects identified if they occurred irregularly or in small numbers. When more than one species occurred within a genus it was usually impossible to relate contamination with soft rot coliforms to individual species.

The main groups of insects collected from both sites were similar to those found in 1973, and of the 8 genera identified from Burrelton, only *Coenosia* was not found at Perth. Four genera, *Parascaptomyza*, *Azelia*, *Nemopoda* and *Nupedia*, occurring at Perth, were absent from Burrelton. There was no appreciable difference in insect activity at each site, probably resulting from the dumping of fresh potatoes at both places, so that rotting tubers were always present.

The combined data from Perth and Burrelton show that of 1878 insects tested, 84 (4.5%) were contaminated with soft rot coliforms. *Scatophaga* spp. and *Delia* spp. were the most frequently contaminated, but they represented only 1.9% of the insects caught. *Leptocera* spp. were again the most frequently collected insects representing 52% of those tested. They yielded 32.1% of the *Erwinia* isolates, but only 2.8% of them carried the bacteria. *Drosophila* spp. were the second most frequently collected insects (12.9%); they yielded 33.3% of the bacterial isolates. *Sepsis* spp., *Themira* spp. and *Nemopoda nitidula* represented 7.6% of the insects tested and accounted for

Table 5. Contamination by *Erwinia* sp. of insects collected from Perth, June-November 1974.

	Number collected[1]	Percentage of total insects collected[2]	Number contaminated with *Erwinia*[3]	Percentage contaminated with *Erwinia*[4]	Percentage of total number of *Erwinia* isolates[5]
Azelia sp.	21	2.2	4	19.1	8.7
Delia spp.	7	0.7	2	28.6	4.3
Drosophila spp.	70	7.3	9	12.9	19.6
Leptocera spp.	563	58.6	18	3.2	39.1
Nemopoda nitidula	32	3.3	3	9.4	6.5
Nupedia infirma	5	0.5	1	20.0	2.2
Parascaptomyza pallida	1	0.1	1	100.0	2.2
Scatophaga squalida	3	0.3	2	66.7	4.3
Scatopse spp.	19	2.0	0	0.0	0.0
Sepsis spp.	68	7.1	4	5.9	8.7
Themira minor	5	0.5	1	20.0	2.2
Other Diptera[6]	157	16.3	1	0.6	2.2
Other insects[7]	10	1.1	0	0.0	0.0

[1 – 7] *Siehe Tabelle 2 – Voir tableau 2*

Tabelle 5. Kontamination von in Perth gesammelten Insekten mit *Erwinia* sp., Juni-November 1974.
Tableau 5. Contamination par *Erwinia* sp. d'insectes capturés à Perth, juin-novembre 1974.

Table 6. Contamination by *Erwinia* sp. of insects collected from Burrelton, June-November 1974.

	Number collected[1]	Percentage of total insects collected[2]	Number contaminated with *Erwinia*[3]	Percentage contaminated with *Erwinia*[4]	Percentage of total number of *Erwinia* isolates[5]
Coenosia tricolor	20	2.2	0	0.0	0.0
Delia florilega	18	2.0	2	11.1	5.3
Drosophila spp.	173	18.8	19	11.0	50.0
Leptocera spp.	414	45.0	9	2.2	23.7
Scatophaga stercoraria	7	0.8	2	28.6	5.3
Scatopse spp.	54	5.9	0	0.0	0.0
Sepsis spp.	28	3.0	2	7.1	5.3
Themira putris	10	1.1	1	10.0	2.6
Other Diptera[6]	195	21.2	3	1.5	7.9

[1 – 6] *Siehe Tabelle 2 – Voir tableau 2*

Tabelle 6. Kontamination von in Burrelton gesammelten Insekten mit *Erwinia* sp., Juni-November 1974.
Tableau 6. Contamination par *Erwinia* sp. d'insectes capturés à Burrelton, juin-novembre 1974.

10.7% of the *Erwinia* isolates. *Azelia* sp. yielded 4.8% of the bacterial isolates although representing just over 1% of the insects tested. Unlike 1973, no members of the genus *Scatopse* were found contaminated. Among the many miscellaneous dipterans were a small number of identified insects which also did not yield soft rot coliforms, namely *Lycoriella nitidicollis*, *Microchrysa polita*, *Neoascia podagrica* and *Piophila vulgaris* from Perth, and *Drapetis humilis* from Burrelton.

Changes in the composition of the insect fauna were observed over the sampling period with the main groups of insects following a pattern similar to 1973. *Leptocera* spp. were present throughout the whole period with peak numbers occurring from late June to mid-August, whereas *Sepsis* spp. appeared in the first collections, reached maximum populations in July and then declined. *Drosophila* spp. were absent until mid-July then became more prevalent as the season progressed. In late September, *Azelia* sp. appeared and were still prominent in November, when *Nupedia infirma* and the 2 species of *Scatophaga* were collected.

Bacteria found in debris from dumps in 1975

The identity, number and ratio of numbers of isolates of *E. carotovora* and *E. atroseptica* obtained from the samples collected at Perth and Burrelton are summarized by date in Table 7, along with information about the kind of debris sampled. No correlation was found at Burrelton between depth of sampling and number of samples yielding soft rot coliform bacteria. At Perth, the results showed that more surface samples yielded soft rot coliform bacteria and that *E. atroseptica* predominated in these samples. As explained later this may reflect the pattern of dumping of potatoes at the site.

At the Perth dump 41.7% of all samples tested yielded soft rot coliform bacteria while only 14.6% were found contaminated at Burrelton. The highest numbers of bacteria occurred at the start of sampling (winter and spring) at each site, declined over the summer period and rose again towards the end of the year. *E. atroseptica* and *E. carotovora* were found on all sampling dates with the exception of 25 August at Perth when only *E. carotovora* was isolated and at Burrelton on 25 August and 8 September when no soft rot coliform bacteria were found. In the samples taken during the early part of the year from both sites *E. atroseptica* predominated, but by 30 June at Perth a change to *E. carotovora* took place which was even more pronounced in the August samples. However by December *E. atroseptica* again became the more frequently isolated organism at Perth. At Burrelton on 30 June, *E. atroseptica* was still more prevalent than *E. carotovora* in a diminishing number of samples yielding soft rot coliforms, but by 13 October *E. carotovora* was by far the most frequently isolated organism. The position remained unchanged in the samples collected on 2 December.

Changes in the ratio of numbers of isolates of *E. carotovora* to *E. atroseptica* observed over the sampling period at both Perth and Burrelton very probably reflected the different pattern of dumping of potatoes at each site. At Perth, *E. atroseptica* predominated in the samples obtained from recently dumped debris (in January,

Table 7. Isolation and ratio of *Erwinia carotovora* var. *carotovora* to *Erwinia carotovora* var. *atroseptica* from debris and soil in dumps by date, 1975.

	Number of samples tested[1]	Number of samples yielding[2]			Number of isolates tested[4]	Ratio of var. *carotovora* to var. *atroseptica*[5]	Remarks[6]*
		var. *atroseptica*	var. *carotovora*	mixed[3]			
Perth							
29. 1.75	27	6	1	8	90	1:2.2	a
28. 4.75	21	9	1	1	54	1:3.9	b
30. 6.75	36	2	4	4	60	1:0.6	c
25. 8.75	36	0	9	0	54	–	c
2.12.75	36	6	9	5	120	1:1.1	d
Burrelton							
3. 3.75	30	7	5	3	86	1:1.3	e
28. 4.75	31	3	1	0	36	1:2.6	f
30. 6.75	45	2	1	0	18	1:2.0	g
25. 8.75	45	0	0	0	–	–	h
8. 9.75	42	0	0	0	–	–	i
13.10.75	27	1	9	0	60	1:0.1	j
2.12.75	27	0	3	1	24	1:0.1	j

[1] *Anzahl untersuchter Muster – Nombre d'échantillons testés;* [2] *Anzahl Muster mit – Nombre d'échantillons atteints par;* [3] *Gemischt – Mélange des deux variétés;* [4] *Anzahl der untersuchten Isolate – Nombre d'isolements testés;* [5] *Verhältnis von var. carotovora zu var. atroseptica – Rapport entre var. carotovora et var. atroseptica;* [6] *Bemerkungen – Remarques*

*a Samples from debris dumped in previous 3 months. Fresh dumping in progress – *Muster von Ueberresten, abgelagert in den vorhergehenden 3 Monaten. Frische Ablagerung im Gang – Echantillons provenant des déchets entassés 3 mois au préalable nouveaux dépots en cours.*

b Samples from debris dumped in January 1975. Areas sampled 29.1.75 covered over by fresh material – *Muster von im Januar 1975 abgelagerten Ueberresten. Stellen, von den am 29.1.75 Muster entnommen wurden, mit frischem Material überdeckt – Echantillons provenant des déchets déposés en janvier 1975, la surface échantillonnée le 29.1.75 a été recouverte par du matériel nouveau.*

c Samples from debris dumped in the period January-April 1975 – *Muster von Abfällen, die in der Zeit vom Januar-April 1975 abgelagert wurden – Echantillons provenant de déchets déposés pendant la période de janvier à avril 1975.*

d 18 samples from areas as 25.8.75 and 18 from debris dumped over previous 2 months (gave var. *carotovora*/var. *atroseptica* ratios of 1:0.4 and 1:1.5, respectively) – *18 Muster von den Stellen wie am 25.8.75 und 18 von Abfällen, die vor 2 Monaten abgelagert wurden (Gab Verhältniswerte von var. carotovora zu var. atroseptica von 1:0.4 bzw. 1:1,5) – 18 échantillons provenant du terrain nu au 25.8.75 et 18 provenant de déchets déposés dessus 2 mois au préalable (cela donne pour la var. carotovora par rapport à la var. atroseptica les ratios suivants: 1:0,4 et 1:1,5 respectivement).*

e 27 samples from area 1 (large bulk of tubers dumped in July 1974) and 3 samples from area 2 (little or no dumping in previous 3 years) – *27 Muster von der Stelle 1 (grosser Knollenhaufen, abgelagert im Juli 1974) und 3 Muster von der Stelle 2 (wenig oder keine Ablagerung in den vorhergehenden 3 Jahren) – 27 échantillons provenant de l'endroit 1 (de gros apports de tubercules ont été déposés en juillet 1974) et 3 échantillons provenant de l'endroit 2 (absence ou faible dépot pendant les 3 années précédentes).*

f 27 samples from area 1 (extensive soft rotting in progress); none yielded *Erwinia* sp.; 4 samples

from area 2 – *27 Muster von Ort 1 (umfassende zunehmende Nassfäule); keine Erwinia sp. gefunden; 4 Mustern von Ort 2 – 27 échantillons provenant de l'endroit 1 (extension des pourritures molles en cours); aucun Erwinia sp.; 4 échantillons proviennent de l'endroit 2.*

g 27 samples from area 1 (breakdown of tubers almost complete – no green stems); none yielded *Erwinia* sp.; 18 samples from area 2 – *27 Muster von Ort 1 (Zerfall der Knollen fast vollständig – keine grünen Stengel); keine Erwinia sp. gefunden; 18 Mustern von Ort 2 – 27 échantillons provenant de l'endroit 1 (altération des tubercules presque complète, pas de tiges vertes); aucun Erwinia sp.; 18 échantillons proviennent de l'endroit 2.*

h 18 samples from area 1 (breakdown of tubers complete – no sign of growth); 27 samples from area 2 – *18 Muster von Ort 1 (Zerfall der Knollen vollständig – kein Anzeichen von Wachstum); 27 Muster von Ort 2 – 18 échantillons provenant de l'endroit 1 (altération des tubercules complètes, aucun indice de croissance); 27 échantillons provenant de l'endroit 2.*

i 15 samples from area 1; 27 samples from area 2 – *15 Muster von Ort 1; 27 Muster von Ort 2 – 15 échantillons provenant de l'endroit 1; 27 échantillons provenant de l'endroit 2.*

j All samples from area 2 – *Alle Muster von Ort 2 – Tous les échantillons provenant de l'endroit 2.*

Tabelle 7. Isolation und Verhältnis von *Erwinia carotovora* var. *carotovora* zu *Erwinia carotovora* var. *atroseptica* von Ueberbleibseln und Boden auf Abfallhaufen nach Datum, 1975.
Tableau 7. Isolement et rapport entre *Erwinia carotovora* var. *carotovora* et *Erwinia carotovora* var. *atroseptica* provenant de déchets et terre sur les tas, en fonction de la date, 1975.

April and December) whereas samples from older material (in June and August) yielded more *E. carotovora* isolates. This effect is well illustrated by the 2 December results (Table 7) when 18 samples each of recently dumped and older material were tested. At Burrelton, the results of the ratio of numbers of isolates of *E. carotovora* to *E. atroseptica* in samples collected in March, April and June showed that *E. atroseptica* predominated. However, by 13 October, the next date when samples yielded soft rot coliform bacteria, the predominant organism was *E. carotovora* and it remained more common in the 2 December samples. No dumping of fresh tubers had taken place at the sampling sites during the autumn and early winter of 1975.

Transmission of soft rot coliform bacteria to potato plants by insects

Three days after the plastic bags and flies were removed from the injured plants. soft rotting was observed on one stem on an area which had been crushed with forceps. Twenty-four hours later, 2 further stems on the injured plants exposed to flies showed soft rotting at sites where the tissue had been gouged out. Pectolytic bacteria isolated from the soft rotted tissue and from small pieces of injured tissue exposed to the flies but not showing soft rot symptoms were identified as *E. carotovora* (the organism found to be associated with 11.4% of the flies collected at Perth on the same day as those used in the transmission experiments). Isolates of *E. carotovora* were obtained from 4 of the 5 injured plants exposed to the insects. No soft rot coliform bacteria were isolated from damaged tissue taken from plants not exposed to the flies.

These results show that insects collected at the Perth potato dump readily transmitted *E. carotovora* to damaged areas on the aerial parts of potato plants.

Discussion

There is now a body of evidence that soft rot coliform bacteria do not survive long in non-sterile field soil in temperate climates (for instance see Graham, 1958; Lazar & Bucur, 1964; Logan, 1969) which has raised many important questions about the sources and means of spread of these organisms in the environment. The occurrence of soft rot infections in plants propagated from true seed and plants propagated from material tested and found free from infection suggests that the causal organisms have efficient means of dissemination from overwintering sites.

Studies of soft rot infections on tested potato stem cuttings have demonstrated that dipterous insects dispersing from a waste dump probably transmitted the bacteria to the above-ground parts of plants (Graham et al., 1976). Data presented in this paper again suggest that dipterans are important agents for introducing soft rot coliforms to pathogen-free potato stocks. *Erwinia* contamination of insects may not be uncommon. Of the 12 genera yielding *Erwinia* isolates, only 2 have previously been reported to be associated with these organisms; *Drosophila* spp. (Graham & Hardie, 1971) and *Leptocera* spp. (Graham et al., 1976). It is, however, difficult to form a firm opinion about the extent to which different genera and species of insects are involved in the spread of soft rot coliforms although it seems that many insects attracted to decaying vegetable matter are potential vectors. This is illustrated by the fact that several kinds of dipterans, namely coprophilous genera (e.g. *Leptocera*, *Scatopse*), fruit flies (e.g. *Drosophila*, *Parascaptomyza*) and muscids (e.g. *Coenosia*, *Azelia*) were all found associated with soft rot coliforms, in a limited area of Scotland.

Contaminated insects have been shown, both in the present studies and in others (Molina et al., 1974), to transmit soft rot *Erwinia* spp. readily to injured potato plants. Injury to potato haulm is common and the importance of insects as vectors probably depends on their being attracted to injured tissue rather than lack of damage through which infection might take place. In this connection Graham et al. (1976), observed insects such as *Leptocera* spp. and *Drosophila* spp. visiting damaged stem tissue in the field. In a wet climate where injuries can remain open for relatively long periods, the time factor is probably not critical, because wounds could be susceptible for up to several days, before healing took place. In drier climates where injuries dry rapidly, damaged areas may be susceptible for only a short time. The possibility that *Erwinia* sp. deposited by insects on undamaged tissue may survive until injuries occur needs further investigation.

Potato dumps are often situated in areas where large quantities of seed potatoes are grown. Thus the distances from sources of bacteria and vectors to plants are short, and as already suggested by Graham et al. (1976), accumulation of waste potatoes should be avoided as a general hygienic measure. However, the proximity of potato dumps cannot explain infection of potato crops in isolated areas, or infections in other crops or ornamentals in areas far away from potato production. At least part of this infection might result from contaminated dipterans flying relatively long distances, as discussed by Graham et al. (1976). The ability of the bacteria to survive in

and/or on insects must be a significant factor affecting trasmission. Although methods used in the present study did not allow determination of where the bacteria were located it is probable that they remain viable for some time, and, in some species at least, may re-emerge in vomit drops. The nature of the association between bacteria and insects requires much more study.

The predominance of *E. carotovora* among the isolates from insects caught at both dumps was unexpected, because, in Scotland at least, it had already been established in many experiments that *E. atroseptica* and *E. carotovora* were associated with tubers in the ratio of about 4:1 (Perombelon, 1973; Graham, unpublished). It was therefore thought that insects would be contaminated with these organisms in roughly the same proportion. Investigations of the populations of soft rot coliforms in dump debris showed that the ratio of the organisms changed as tubers decomposed, and during the summer and early autumn, when insect activity was greatest, *E. carotovora* predominated unless new tubers or potato haulm were deposited. Thus, *E. carotovora* is clearly able to survive longer in debris, and it has already been shown that *E. carotovora* can remain viable longer in ordinary soil and at higher temperatures than *E. atroseptica* (for example, see Lazar & Bucur, 1964). The bacteria associated with the insects probably partly reflected the changes in bacterial populations, but it must be remembered that the detailed insect studies were made in 1973 and 1974, whereas investigations on dump debris were carried out in 1975. The ratios are thus not strictly comparable, although a less detailed study of insects caught in 1975 at the sites gave similar results to those obtained previously. However, it is particularly noteworthy that the ratio of *E. carotovora* to *E. atroseptica* was generally higher in insects than in debris, suggesting that insects themselves also exerted some kind of selective action in favour of *E. carotovora*.

The observation that *E. carotovora* predominated in association with insects may help to explain why this organism is more often implicated in soft rot coliform infections in many susceptible plants and plant parts. Whereas *E. atroseptica* is the predominant organism in ordinary commercial potato stocks in Scotland (Graham & Dowson, 1960; Perombelon, 1973), soft rot coliform infections in other plants are nearly always caused by *E. carotovora*.

Although the data support the view that the role of insect vectors in the epidemiology of bacterial soft rots is important, other means of aerial transmission cannot be discounted. Graham & Harrison (1975) showed that raindrops falling on potato stems affected by blackleg could generate aerosols of *E. atroseptica* which could move readily in air streams. Airborne spread of aerosolised soft rot coliform bacteria is being given more study to determine the significance of this method of transmission in the epidemiology of soft rot diseases.

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Zusammenfassung

Kartoffelabfälle als Kontaminationsquelle von Insekten mit koliformen Nassfäulebakterien und die Wiederverseuchung von Kartoffelbeständen, die frei von pathogenen Organismen waren

Zwölf Arten von Insekten der Gattung Diptera, periodisch gesammelt auf zwei grossen Kartoffelabfallhaufen in Schottland, waren mit koliformen Nassfäulebakterien kontaminiert (Tab. 2, 3, 5 und 6). Nur zwei der Arten galten vorher als mögliche Überträger dieser Organismen. 1973 waren 4,6% der an den beiden Orten während der Saison gesammelten Insekten mit diesen Organismen behaftet, 1974 waren es 4,5%. Nicht weniger als 9,6% der Insekten waren bei einigen Fängen im Jahre 1973 und 14,7% im Jahre 1974 Träger von Nassfäule *Erwinia* sp.

Die Fruchtfliegen (*Parascaptomyza* sp. und *Drosophila* spp.) waren 1973 die am häufigsten kontaminierten Insekten (Tab. 2 und 3). Diese Gruppe von Insekten war auch 1974 meistens kontaminiert, aber am häufigsten wurden die Bakterien von *Scatophaga* spp. und *Delia* spp. (Tab. 5 und 6) isoliert.

Sowohl 1973 als auch 1974 wurde *Erwinia carotovora* var. *carotovora* (in der Folge *E. carotovora* genannt) häufiger von den Insekten isoliert als *Erwinia carotovora* var. *atroseptica* (in der Folge *E. atroseptica* genannt) (Tab. 1 und 4), obwohl die Knollen, die ursprünglich den Abfallhaufen ausmachten, fast sicher meistens mit *E. atroseptica* infiziert waren. Das Verhältnis von *E. carotovora* zu *E. atroseptica*, isoliert von Insekten beider Haufen, war 1973 9,5:1 (13,5:1 und 5,5:1 je Abfallhaufen) und 1974 3,8:1 (2,7:1 und 6,8:1 für die beiden Orte). *E. atroseptica* wurde 1973 nur früh in der Vegetationszeit (vor dem 30. Juli) von den Insekten isoliert (Tab. 1), 1974 nur früh (vor dem 30. Juli) oder spät in der Vegetationszeit (nach dem 10. Oktober) (Tab. 4). *E. carotovora* dagegen konnte in beiden Jahren über die ganze Vegatationszeit isoliert werden (Tab. 1 und 4). Die Wiedergewinnung von *E. atroseptica* von Insekten war mit dem Vorhandensein von frisch abgelagerten faulen Knollen zur Zeit, wenn die Insekten gesammelt werden, verbunden. Einzig *E. carotovora* wurde von Insekten isoliert, die zu einer Zeit gesammelt wurden, wenn auf den Abfallhaufen nur älteres verfaulendes Material vorhanden war.

Isolationen von faulenden Ueberresten, die 1975 dem Abfallhaufen entnommen wurden (Tab. 7), zeigten, dass sowohl *E. carotovora* als auch *E. atroseptica* in der Regel das ganze Jahr vorhanden waren. *E. atroseptica* herrschte früh im Jahr (Januar bis Juni) vor und an einem Ort auch spät in der Zeit der Musterentnahme (Oktober bis Dezember). *E. carotovora* war von Juni bis Oktober der am meisten isolierte Organismus. Das Vorherrschen von *Erwinia carotovora* var. *atroseptica* war mit dem Vorhandensein von frisch weggeworfenen Kartoffelknollen früh und spät in der Zeit der Musterentnahme verbunden. *E. carotovora* schien in den faulenden Knollen länger zu überleben und war der am meisten isolierte Organismus aus älterem verwesendem Material.

Die Verhältniswerte von *E. carotovora* zu *E. atroseptica*, isoliert von Insekten, waren höher als jene, die für das verfaulende Material aus den Abfallhaufen gefunden wurden. Es ist anzunehmen, dass die Insekten selbst einen selektiven Einfluss zu Gunsten von *E. carotovora* ausüben.

Von einem der Abfallhaufen gesammelten Insekten übertrugen im Glashaus *E. carotovora* sogleich auf verletzte Kartoffelstengel. Man nimmt an, dass Kartoffelbestände, die mit Hilfe des Stengelschnittes von koliformen Nassfäuleerregern befreit wurden, durch Insekten, die von Kartoffelabfallhaufen kommen, wieder verseucht werden können.

Résumé

Bactéries coliformes responsables de la pourriture molle, associées à des insectes

12 genres d'insectes appartenant à l'ordre des Diptères, capturés périodiquement en Ecosse, sur deux grands tas de déchets de pommes de terre se sont révélés être contaminés par des bactéries coliformes, agents de la pourriture molle (tableaux 2, 3, 5 et 6). Auparavant, seuls 2 genres avaient été cités comme vecteurs possibles de ces microorganismes. En 1973, 4,6% des insectes capturés aux 2 endroits, et en cours de saison, portaient ces parasites de la pomme de terre. En 1974, 4,5% étaient également contaminés. Pour l'ensemble de ces captures, il y a eu 9,6% des insectes en 1973, et 14,7% en 1974 qui portaient *Erwinia* sp.

Les mouches des fruits (*Parascaptomyza* et *Drosophila* spp.) ont été le plus fréquemment contaminés en 1973 (tableaux 2 et 3). Ce groupe d'insectes était également atteint en 1974, mais les bactéries on été plus fréquemment isolées à partir de *Scatophaga* spp. et *Délia* spp. (tableaux 5 et 6).

En 1973 et 1974, *Erwinia carotovora* var. *carotovora* (dans la suite *E. carotovora*) a été plus fréquemment isolé de ces insectes qu'*Erwinia carotovora* var. *atroseptica* (dans la suite *E. atroseptica*) (tableaux 1 et 4) bien que les tubercules constituant à l'origine les tas de déchets étaient certainement plus infestés par *E. atroseptica*. Le rapport entre *E. carotovora* et *E. atroseptica* pour les isolements sur insectes provenant des deux endroits a été 9,5/1 (respectivement 13,5/1 et 5,5/1 pour chacun des tas de déchets) en 1973 et 3,8/1 (2,7/1 et 6,8/1) en 1974. *E. atroseptica* n'a été isolé que tôt dans l'année (avant le 30 juillet) en 1973 (tableau 1) et 1974 (tableau 4) elle l'a été précocement (avant le 30 juillet) ou tardivement (après le 10 octobre). *E. carotovora*, par contre, l'a été durant toute la saison pendant les deux années (tableaux 1 et 4) l'isolement d'*E. atroseptica* est lié au moment de la capture des insectes, à la présence de tubercules nouvellement pourris lors de la mise en tas. Par contre *E. carotovora* a été isolé à partir des insectes capturés sur les tas où il n'y avait que du matériel présentant des pourritures plus agées.

En 1975, des isolements effectués sur les tas de déchets ont montré qu'*E. carotovora* et *E. atroseptica* étaient habituellement présents toute l'année (tableau 7). *E. atroseptica* était prédominante en début de saison (de janvier à juin). Il n'y a eu qu'un seul cas où on l'a observé plus tardivement durant la période d'échantillonage (octobre à décembre). *E. carotovora* a été le principal organisme isolé de juin à octobre. La prédominance d'*E. atroseptica* était associée à la présence de tubercules nouvellement entreposés précocement et tardivement durant la période d'échantillonage. *E. carotovora* semblait subsister plus longuement dans les tubercules pourris et était le principal organisme isolé à partir de matériel altéré le plus agé.

Les rapports *E. carotovora* sur *E. atroseptica* obtenus à partir des isolements effectués sur insectes ont été plus élévés que ceux trouvés à partir du matériel pourri en provenance des tas. Les auteurs suggèrent que les insectes eux-mêmes peuvent exercer une action sélective en faveur d'*E. carotovora*.

Les insectes collectés à partir d'un des tas peuvent transmettre facilement *E. carotovora* aux pommes de terre se trouvant en serre et dont les tiges sont endommagées. Les auteurs suggèrent que les lots de pommes de terre indemnes de bactéries coliformes grace au procédé de bouturage peuvent être à nouveau recontaminé à partir des tas de déchets de pommes de terre et par l'intermédiaire des insectes.

References

Boyd, A. E. W., 1974. Sources of potato blight (*Phytophthora infestans)* in the east of Scotland. *Pl. Path.* 23: 30–36.

Graham, D. C., 1958. Occurrence of soft rot bacteria in Scottish soils. *Nature, Lond.* 181: 61.

Graham, D. C. & W. J. Dowson, 1960. The coliform bacteria associated with potato blackleg and other soft rots. 1. Their pathogenicity in relation to temperature. *Ann. appl. Biol.* 48: 51–57.

Graham, D. C. & J. L. Hardie, 1971. Prospects for the control of potato blackleg disease by the use of stem cuttings. *Proc. 6th Br. Insectic. Fungic. Conf.* 1: 219–224.

Graham, D. C. & M. D. Harrison, 1975. Potential spread of *Erwinia* spp. in aerosols. *Phytopathology* 65: 739–741.

Graham, D. C., C. E. Quinn & M. D. Harrison, 1976. Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967–74. *Potato Res.* 19: 3–20.

Johnson, C. G., 1969. In: Migration and dispersal of insects by flight. Methuen, London.

Lazar, I. & El. Bucur, 1964. Recent research in Roumania on blackleg and bacterial soft rot of potato. *Eur. Potato J.* 7: 102–111.

Logan, C., 1969. The survival of the potato blackleg pathogen overwinter. *Rec. agric. Res. Min. Agric. N. Ireland* 17: 115–121.

Molina, J., M. D. Harrison and J. W. Brewer, 1974. Transmission of *Erwinia carotovora* var. *atroseptica* by *Drosophila melanogaster*. 1. Acquisition and transmission of the bacterium. *Am. Potato J.* 51: 245–250.

Perombelon, M. C. M., 1973. Sites of contamination and numbers of *Erwinia carotovora* present in stored seed potato stocks in Scotland. *Ann. appl. Biol.* 74: 59–65.

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Quantitative Studies on the Generation of Aerosols of *Erwinia carotovora* var. *atroseptica* by Simulated Raindrop Impaction on Blackleg-infected Potato Stems

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Quantitative studies on the generation of bacterial aerosols by simulated raindrop impaction on potato stems infected with the blackleg organism *Erwinia carotovora* var. *atroseptica* have shown that, theoretically, airborne transmission might result in re-contamination of blackleg-free potato stocks.

SINCE 1967 the Department of Agriculture and Fisheries for Scotland has produced potato stocks free from infection with certain tuber-borne pathogens including the blackleg organism *Erwinia carotovora* var. *atroseptica* by the use of stem cuttings These stocks are known as VTSC (Virus Tested Stem Cutting) seed and VTSC is the highest grade in the Scottish Seed Potato Certification Scheme. In 1970, VTSC material was first released to specialist growers and by 1976 a very large part of the seed crop in Scotland was grown from stem cutting progeny. Eventually all seed potatoes will be derived from VTSC material and it is therefore very important to prevent these stocks from becoming re-contaminated with soft rot *Erwinia* spp.

Details of the methods of VTSC production and some early experiences with VTSC material are described by Graham & Hardie (1971). Observations indicated that low levels of re-contamination with soft rot coliforms occurred, which could build up rapidly under favourable environmental conditions. Since 1970 research into how re-contamination takes place has centred on two fields of study. First, it has been shown that several species of insects act as carriers of soft rot coliform bacteria and can transmit them to damaged potato stems (Graham *et al.* 1976; Harrison *et al.* 1977); secondly it has been demonstrated that airborne transport of the potato blackleg organism could occur following the generation of aerosols by raindrop impaction on infected stem tissue.

Graham & Harrison (1975) built a combined raindrop simulator/wind tunnel which enabled them to show that when raindrops fell on to infected potato stems, an aerosol of viable blackleg organisms was produced, probably by a combination of bursting bubbles (Blanchard & Syzdek 1970) and a coronet-shaped upward surge of droplets (Darlow 1972). The aerosol moved readily in slow air streams and remained airborne for 60–90 min. However, these experiments gave no quantitative information about the number of viable aerosol propagules generated from unit weight of infected stem.

Investigations described here were aimed at determining the number of viable propagules produced from infected stems in a variety of circumstances. The ability of 3 and 5 mm diam. raindrops to generate aerosols was compared; the effect of length of incubation of infected stems on the number of viable propagules released from them was determined; and several potato varieties were compared as sources of bacteria. These data were then used to calculate the theoretical number of propagules that could be released into the air from a blackleg-infected crop, and hence the number that could fall on unit area at unit distance down wind of crops under standard meteorological conditions. This information would help in judging the potential significance of aerosol transmission as a way that potato crops could be re-contaminated, and if the data showed airborne transmission could be important, indicate possible separation distances between crops that would be necessary to minimize re-contamination. The quantitative data on aerosol generation were obtained using the apparatus built by Graham & Harrison (1975) but with certain modifications.

Materials and Methods

Apparatus for generating aerosols

The apparatus illustrated by Graham & Harrison (1975), consisted of a plastic tube (15·2 cm i.d., 7·6 m high) from the top of which water drops were released from a reservoir. The drops fell on to infected stem tissue placed on the surface of moist sterilized soil in a shallow plastic tray, inside a stainless steel chamber.

The chamber, which had an air-tight access door, was large enough to contain any splash droplets. It was supplied with humidified air (90–95% r.h. at 10·6 °C), using an adapted commercial humidifier (Xpelair EH10 Humidifier, GEC-Expelair Ltd, Witton, Birmingham). The air was first drawn into the chamber through a duct from the humidifier, then out along a wind tunnel made of 38 cm diam. aluminium tubing 2·7 m long, by a 30·5 cm diam. extractor fan fitted at the far end of the tunnel. The fan motor was controlled through a rheostat, adjusted to give a slow wind speed. In these experiments the air flow in the wind tunnel was set to give a mean velocity of 23 m/min, measured with a thermocouple air flow meter (Davimeter, Airflow Developments Ltd, High Wycombe, Buckinghamshire).

The air was drawn through a sampling tube 4 cm i.d., located in the centre of the wind tunnel near its end, 4 m from the target area of the falling drops. The original apparatus used by Graham & Harrison (1975) had a sampling tube of only 1 cm diam., and as the velocity of the air in the tube was greater than the velocity of air in the wind tunnel, sampling was anisokinetic, so that the number of viable propagules in unit volume of air in the tunnel could not be calculated. The larger intake tube allowed the velocity in the tunnel to equal the velocity in the tube, so that sampling was isokinetic, and the number of viable propagules in the air could be determined (May 1967). The inside of the sampling tube was polished to assist smooth air flow, and the leading edge machined down to a width of less than 1 mm, to minimize particle impaction.

By the time the falling water drops reached the stems, those of 3 mm diam. had achieved 97% of terminal velocity and those of 5 mm diam. had achieved 94% of terminal velocity, so that they simulated raindrops (Gregory *et al.* 1959). Examination of the bombarded stem pieces showed that all the tissue had been struck by the drops.

Samplers, media and sampling

Sampling with Casella samplers

A 3·2 cm diam. flexible plastic pipe 50 cm long connected the sampling tube alternately to Casella airborne bacteria samplers, drawing air at the rate of 30 l/min. To prevent build up of any electrostatic charge on the walls of the connecting pipe which could have affected the sampling by attracting particles to the inner surface, the inside was treated with an antistatic polish (ICI Perspex polish No. 3). To reduce any possible loss by wall impaction of the aerosol particles on bends in the pipe and also to allow ease of change-over from one sampler to another, the samplers were inclined (at an angle of 45°) on wooden supports. At this angle, it was necessary accurately to fix and centre the 9 cm diam. plastic Petri dishes (used in culturing the bacteria in the particles) to the sampler turntable. Two 1·25 cm wide strips of double-sided adhesive tape were attached in parallel to the base of each plate, which was also marked at the centre with a cross. The centre of the turntable base was marked with a cross around which two semicircular pads of 1·2 cm thick plastic foam were fixed. Each Petri dish was positioned by aligning the crosses and pressing the base on to the foam.

In preliminary experiments it had been found that aerosol was released for more than 30 min after raindrops began to fall and samples collected from the wind tunnel over this period on one Petri dish containing a suitable medium produced far too many colonies to count. Therefore two samplers had to be used alternately, so that while one machine was sampling, the Petri dish in the other could be replaced with a fresh one. The break in sampling while the connecting pipe was being changed from one sampler to the other was less than three seconds, and the small loss of aerosol during the change-over was neglected. Thus, the sample from each stem was collected on a series of Petri dishes as described below.

The first sampling experiments showed that most pectolytic colonies grew on the medium exposed at the beginning of rainfall, and that there was usually a decline in the numbers on the later Petri dishes in the series. It was found empirically that the best sequence of exposure times for plates in tests using drops 3 mm in diam. was 2, 2, 5, 5, 5, 5 and 5 min; and for 5 mm diam. drops, 0·5, 0·5, 2, 2, 2, 5, 5, 5, 5 and 5 min. Although the aerosols appeared to be generated more rapidly when 5 mm drops bombarded the stems, it was notable that irrespective of whether 3 mm or 5 mm drops were employed, the majority of the colonies grew on the plates used to collect the aerosol given off during the first 20 min of raindrop bombardment.

At the end of each experiment the raindrops were stopped and a control plate was run for 2 min to ensure the system was clear of viable bacterial propagules. If any colonies developed on this plate their number was added to the total plate count for the series.

Media

The particles containing blackleg bacteria were deposited on Stewart's (1962) double layer MacConkey-pectate gel medium (prepared from Oxoid CM7 MacConkey medium) in the Petri dishes, which were incubated at 26 °C for 48 h and the characteristic pectolytic colonies counted. Perombelon & Lowe (1971) indicated that this medium partly inhibited the growth of *E. carotovora* var. *atroseptica*, and studies on the survival of bacteria in aerosols have shown that bacterial cells can be damaged in several ways such as by the process of aerosolization itself, exposure to oxygen in the air, and desiccation-rehydration processes (*vide* Hambleton & Benbough 1973). It was

considered that the bacteria in the aerosol might be in a physiological state where many of the cells might not grow on a selective medium whereas they might grow on a non-selective one, such as nutrient agar. However, 14 tests comparing these two media showed that although Stewart's medium was somewhat less efficient than nutrient agar in detecting viable propagules there were no substantial differences between them, and Stewart's medium was much easier to use as the colonies of *Erwinia* could be counted directly. There were many bacterial and some fungal contaminants on the nutrient agar in Petri dishes, so that the soft rot coliform colonies were detected by replica plating (after 48 h incubation) on to Stewart's medium from the nutrient agar.

Sampling with the Andersen sampler

To determine the size distribution of the aerosol particles, an Andersen sampler (Andersen 1958) was connected to the sampling tube of the aerosol generator using the same plastic pipe as the Casella samplers. Because the air was drawn into the sampler at the rate of 28·3 l/min, sampling was approximately isokinetic. The plastic Petri dishes used in the sampler were coated externally with Perspex antistatic polish to prevent any electrostatic deposition of the aerosol particles. Stewart's medium was also employed in this sampler for culturing bacteria in the aerosol.

Preparation of inoculum and production of infected stem tissue

The stem tissue used in the experiments was taken from potato plants grown in pots (25 cm diam.) in the greenhouse. About four weeks after emergence, the stems (roughly 1 cm in diam.) were cut and inoculated after removing the leaves. Inoculum was prepared by washing the growth off three agar slopes of a 1–2 d old culture of a typical virulent *E. carotovora* var. *atroseptica* isolate (strains G125, G309 or G345) using 3 ml of sterile water. This produced sufficient suspension to inoculate a 20–25 cm length of potato stem and ensure adequate rotting. A Pasteur pipette was used to introduce the inoculum into the stems through several longitudinal slits made with a scalpel blade.

Stems were placed on damp filter paper in a Perspex humidity chamber, and incubated at 26 °C. After 1–5 d, lengths were cut, weighed and used in the experiments as described below. Usually the stems were well rotted in 2–3 d.

Determination of the effect of the length of incubation period on the number of bacterial aerosol propagules produced from infected stems as compared with the total number of bacteria in the tissue

To determine the efficiency of generation of bacterial propagules from infected tissue by raindrop impaction as affected by length of incubation period, the total number of bacteria in infected tissue was compared with the number of propagules released as an aerosol each day for 5 d after inoculation.

Five potato stems, var. *Majestic*, each *ca.* 25 cm long and of similar thickness and texture were inoculated as described previously. On days one and five after inoculation, four sections, 3 cm long were cut from one stem. The pieces were then sliced in two longitudinally, and halves from each adjacent piece of stem were placed together and weighed. Two of these composite sections were used for determination of total number of bacteria present, the others for aerosol generation. By using halves from different parts along the stem, errors caused by uneven rotting were reduced, although rotting appeared to be reasonably uniform. On days two, three and four after inoculation an

additional three stem pieces were halved, paired, weighed and used for aerosol generation.

To determine the number of bacteria in the stem sections, the two lengths of composite stem pieces were macerated separately in 5 ml sterile water for 2 min in an MSE homogenizer. Ten-fold serial dilutions were prepared from the resulting suspensions; these were agitated for 0·5 min using a Fisons 'Whirlimixer'. Samples of 0·2 ml of each dilution were pipetted on to the surface of Stewart's medium in each of three Petri dishes and distributed by carefully rotating the plates by hand. The Petri dishes were incubated for 48 h and the colonies then counted (usually the 10^5 or 10^6 dilutions).

For aerosolization experiments, water drops 5 mm in diam. falling at the rate of 160 drops/min were used. Rotting stems were laid on moist soil in a plastic tray which was placed in the chamber but not immediately under the target area of the falling drops. The humidifier was switched on, the drops were started and their rate checked with a stop watch. The stem material was then positioned in the target area and the chamber door shut. The sampler and then the tunnel extractor fan were switched on. At the end of the sampling time the drops were stopped and the control plate run.

Determination of the number of bacterial aerosol propagules containing Erwinia carotovora *var.* atroseptica *produced from stems of different potato varieties*

The efficiency of aerosol production was investigated using 76 stem pieces of six varieties (of varying field susceptibility to blackleg) in tests with drops 5 mm in diam. and 18 stem pieces of two varieties in experiments with drops 3 mm in diam. In all cases stems were 20–25 cm long and incubated 2–3 d after inoculation before use. Serial dilution experiments were also carried out on a proportion of the stems so that the number of aerosol propagules could be compared with the total number of viable bacteria present in the tissue.

Determination of the particle size distribution of aerosol propagules

Stems of two potato varieties were used 3 d after inoculation. 5 mm drops were allowed to fall on to each of five 3 cm lengths of stem for a 5 min sampling period using the Andersen sampler. After incubation for 48 h, the colonies were counted, and a standard correction factor applied to allow for the possibility of more than one organism passing through the same hole in the sampler sieve plate. The tests were repeated with 3 mm diam. drops using each of four 3 cm lengths of stem.

Results

Effect of length of incubation time on generation of bacterial aerosols from infected potato stems

The experiments gave the number of *E. carotovora* var. *atroseptica* colonies developing from the aerosol propagules generated from 1 g of stem tissue. Two examples of the plate counts are given in Tables 1 and 2. Results comparing the number of aerosol propagules with the number of viable bacteria in tissue are given in Fig. 1. This shows that 2 d after the introduction of the inoculum into the stems, the number of bacteria reached a peak within the tissue. There was a similar peak in the number of viable aerosol propagules generated by raindrop impaction. The number in the tissue then declined as the incubation period lengthened, and the number of aerosol propagules generated fell correspondingly.

In subsequent experiments, infected stem tissue was used when large numbers of viable blackleg bacteria were present (probably at the end of the logarithmic growth phase) and could produce many aerosol propagules. Impaction of 5 mm diam. drops produced larger numbers of aerosol particles containing viable bacteria than 3 mm diam. drops but the difference was relatively small. It is noteworthy that raindrop impaction was not a particularly efficient way of generating aerosols as only about 0·003% of viable propagules was produced as compared with the number of bacteria available for aerosolization.

TABLE 1

Plate counts of numbers of aerosol propagules of Erwinia carotovora *var.* atroseptica *released from stems one day after inoculation (5 mm diam. drops)*

Plate No.		1	2	3	4	5	6	7	8	9	10	Control	Total
Sampling time (min)		0·5	0·5	2	2	2	5	5	5	5	5	2	34
Plate count	Stem A	97	44	32	5	3	8	4	5	8	5	2	213
	Stem B	30	21	27	10	7	5	1	3	8	7	3	122

TABLE 2

Plate counts of numbers of aerosol propagules of Erwinia carotovora *var.* atroseptica *released from stems two days after inoculation (5 mm diam. drops)*

Plate No.		1	2	3	4	5	6	7	8	9	10	Control	Total
Sampling time (min)		0·5	0·5	2	2	2	5	5	5	5	5	2	34
Plate count	Stem A	57	41	117	25	11	13	8	1	2	1	0	276
	Stem B	53	58	196	49	23	26	21	12	8	9	1	456
	Stem C	91	115	256	68	74	89	102	87	100	52	5	1039
	Stem D	95	52	102	26	59	41	50	48	106	85	3	667
	Stem E	186	97	148	51	41	34	15	10	8	10	2	602

Generation of aerosol propagules from stems of different potato varieties

Average values corrected to 1 g of stem are given in Table 3. Apart from the result using Golden Wonder (a variety that has been found to be less susceptible than the others to blackleg in the field), data for the number of *Erwinia* aerosol propagules produced by both simulated raindrop sizes were fairly consistent and there was little difference between potato varieties as aerosol sources.

Particle size distribution of aerosol propagules

The size distributions of the airborne *Erwinia propagules produced by the two different* sizes of drops were very similar (Table 4) and it appears that, in general, the size of aerosol particle produced does not depend on the size of raindrop over the range 3–5

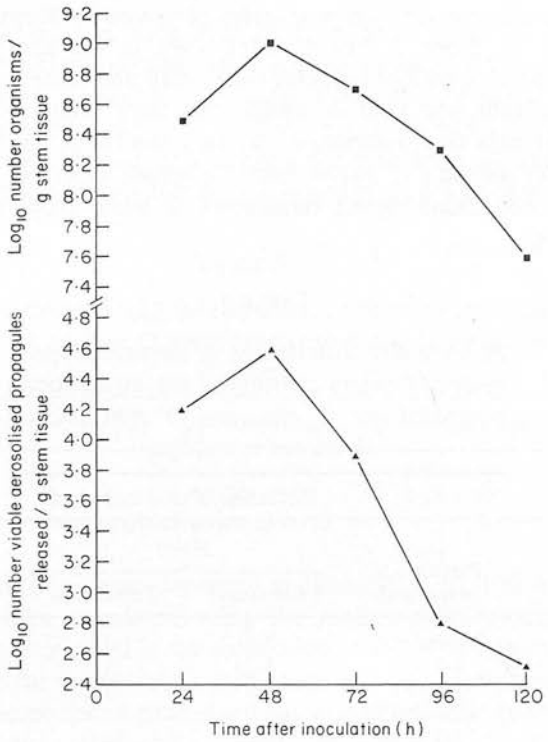

Fig. 1. Comparison of number of aerosol propagules released from (▲) and the number of bacteria contained in (■) 1 g of stem tissue.

TABLE 3

Comparison of numbers of viable bacteria in and number of aerosol propagules generated from 1 g of stem of several potato varieties using simulated raindrops 3 mm and 5 mm in diam.

Potato variety	Total count		Aerosol count	
	No. of stem pieces	No. of *Erwinia* bacteria/g stem	No. of stem pieces	No. of *Erwinia* propagules/g stem
		Drop size: 3 mm in diam.		
Doon Star	6	$9{\cdot}81 \times 10^8$	8	$8{\cdot}04 \times 10^3$
Arran Victory	4	$5{\cdot}92 \times 10^8$	10	$7{\cdot}57 \times 10^3$
		Drop size: 5 mm in diam.		
Epicure	1	$3{\cdot}20 \times 10^8$	10	$2{\cdot}27 \times 10^4$
Majestic	6	$1{\cdot}98 \times 10^9$	34	$2{\cdot}87 \times 10^4$
Golden Wonder	2	$5{\cdot}74 \times 10^8$	3	$5{\cdot}74 \times 10^3$
Arran Victory	6	$4{\cdot}66 \times 10^8$	14	$1{\cdot}19 \times 10^4$
Dunbar Standard	0	—	12	$2{\cdot}99 \times 10^4$
Doon Star	2	$1{\cdot}02 \times 10^9$	3	$1{\cdot}63 \times 10^4$

mm; 92·9% of the airborne *Erwinia* propagules generated by drops 5 mm in diam. and 90·75% generated by drops 3 mm in diam. were in the size range 1·1–4·7 μm. Therefore the majority of aerosol particles were small and some probably consisted of individual *Erwinia* cells, the fluid in which they were originally suspended having evaporated. The particle size distribution was very similar to that given by Venette & Kennedy (1975) for aerosols of *Pseudomonas glycinea* generated from infected soya beans under field conditions during rainstorms or when crops were irrigated with overhead sprinklers.

TABLE 4

Particle size distribution of aerosol propagules of Erwinia carotovora *var.* atroseptica *generated by 3 mm and 5 mm diam. simulated raindrops*

Particle size range (μm)	Percentage of total number of *Erwinia* propagules deposited on plates	
	3 mm drops*	5 mm drops
7·0 and over	2·5	1·6
4·7–7·0	5·25	4·7
3·3–4·7	28·5	27·3
2·1–3·3	44·0	44·6
1·1–2·1	18·25	21·0
0·65–1·1	1·5	1·5

* For drop size 5 mm diam., the values given are the average of results from five stem pieces and for 3 mm diam. drops, results are the average of four stem pieces.

Calculation of deposition gradients from quantitative data for interpretation of gradients in terms of separation distances between crops to avoid re-contamination

The mathematical model described by Gregory (1961) may be used to determine the number of organisms deposited at unit distance down wind from a source, provided the source strength is known (parameter Q_0).

Because sampling was isokinetic, data were obtained for the number of aerosol particles containing blackleg organisms/g of stem tissue produced by rain impaction under optimum conditions. Therefore the source strength for a given percentage of blackleg infection could be calculated for any size of crop, and thus upper limits for the numbers of viable propagules transported over a given distance could be determined, which would give an indication of possible safe separation distances between crops.

The results can be best presented by considering a hypothetical field situation using 2 and 10% levels of stem infection, as illustrated by the following example (these levels are representative of the numbers of infected plants found in commercial potato crops).

In a 0·81 hectare (2 acre) field containing 40 000 plants each with three stems, there is a total number of 120 000 stems. Assuming that half an infected stem is rotted (length of half stem, 25 cm) and the weight of rotted tissue is 11·5 g, then the total weight of

rotted stem can be calculated for each infection level. Source strength (Q_0) is the total number of bacterial aerosol propagules, which is equal to the total weight of rotted stem multiplied by the number of aerosol propagules produced/g of stem. The number of propagules generated/g of stem is $7{\cdot}76 \times 10^3$ for 3 mm drops and $2{\cdot}38 \times 10^4$ for 5 mm drops; these figures were obtained by averaging the data from all aerosol generation experiments. For the two levels of infection, the values of Q_0 are given in Table 5.

TABLE 5

Source strength (Q_0) for two levels of blackleg infection in a 0·81 hectare (2 acre) crop; raindrops 3 and 5 mm in diam.

Level of infection (%)	Q_0—3 mm drops	Q_0—5 mm drops
2	$2{\cdot}14 \times 10^8$	$6{\cdot}57 \times 10^8$
10	$1{\cdot}07 \times 10^9$	$3{\cdot}28 \times 10^9$

The number of viable propagules deposited at distances of 10, 100, 1000 and 10 000 m down wind were then calculated using the mathematical procedures given by Gregory (1961); parameters used in the calculations were: p, the deposition coefficient = 0·001; m, the value for degree of air turbulence = 1·75 (overcast and steady wind speed); h, height of source above ground = 0 m; w, width of field (when considered as an area source) = 100 m.

When calculating deposition values at a given distance from source, depletion of the cloud of airborne particles due to previous deposition must be taken into account, giving a new value for the cloud concentration (parameter Q_x). The correction factor is Q_x/Q_0, the fraction of the initial number of particles still airborne. The correction values were obtained from a standard graph for each distance given by Gregory (1961). From the graph for $p = 0{\cdot}001$, deposition gradients were calculated considering the source as an

TABLE 6

Numbers of aerosol propagules deposited/cm² down wind when the aerosol source is considered as an area and a point

Source	Level of infection (%)	Number of propagules deposited at distances (m) down wind from source			
		10	100	1000	10000
		Drop size: 3 mm in diam.			
Area	2	$3{\cdot}1 \times 10^6$	$7{\cdot}6 \times 10^6$	$5{\cdot}8 \times 10^5$	$3{\cdot}4 \times 10^4$
	10	$1{\cdot}5 \times 10^7$	$3{\cdot}8 \times 10^7$	$2{\cdot}9 \times 10^6$	$1{\cdot}8 \times 10^5$
Point	2	—	$3{\cdot}8 \times 10^2$	1·8	—
	10	—	$1{\cdot}9 \times 10^3$	9·1	—
		Drop size: 5 mm in diam.			
Area	2	$9{\cdot}5 \times 10^6$	$2{\cdot}3 \times 10^7$	$1{\cdot}8 \times 10^6$	$1{\cdot}0 \times 10^5$
	10	$4{\cdot}7 \times 10^7$	$1{\cdot}2 \times 10^8$	$8{\cdot}8 \times 10^6$	$5{\cdot}2 \times 10^5$
Point	2	—	$1{\cdot}2 \times 10^3$	5·6	—
	10	—	$5{\cdot}8 \times 10^3$	27·9	—

area and as a point. Table 6 gives the values for the numbers of *Erwinia* aerosol propagules deposited/cm^2 at given distances down wind from the source.

Discussion

Natural raindrops vary in size up to a maximum diam. of 5 mm, found in thunder rain, but frontal rain contains predominantly smaller drops (Best 1950). However, the results indicate that in a field situation where a crop is affected by blackleg, bombardment by frontal rain could still probably produce large numbers of bacterial propagules, as Graham & Harrison (1975) have shown that 2 mm diam. drops falling under gravity alone possessed enough kinetic energy to generate aerosols, and wind could accelerate these small drops above terminal velocity.

As well as acting as an agent for production of airborne particles, rain could be involved with their deposition, as raindrops collect (or 'wash-out') airborne particles. Efficiency of collection depends on the diameter of the raindrop and the size of the airborne particle; it decreases with decreasing particle size, e.g. when raindrops are 2 mm diam. and spheres 12 μm in diam., the efficiency of collection is *ca.* 75%; with spheres 4 μm in diam., the efficiency is only *ca.* 15% (Chamberlain 1967). To discover if wash-out by rain was likely to reduce the number of airborne particles containing *Erwinia* cells, the particle sizes were determined. In addition, the parameter p used in the formula to calculate expected deposition of airborne particles down wind from the source is related to particle size.

Because of the small size of most of the aerosol particles the efficiency of collection by raindrops would be low. However, it does not follow that most bacteria were present in the 1·1–4·7 μm diam. fractions, because of the much greater volume of the larger particles and the probability that the larger the volume of the particle, the more bacterial cells it will contain (volume of a sphere 2 μm diam. is 4·19 μm^3; volume of a 7 μm sphere is 179·6 μm^3). Thus the larger particles may be more important as sources of bacteria, but more liable to wash-out, although somewhat less liable to desiccation.

Regarding the deposition of airborne bacteria, the numbers of aerosol particles deposited initially decreases rapidly with increasing distance from the source, but as distances increase deposition gradients flatten out. Whether the infected potato crop is considered a point source or an area source is related to the distance from the source; a field could be regarded as a point source when distances down wind are many times the width of the field. When the crop is considered as a point source the numbers of *Erwinia* propagules deposited/cm^2 are much smaller than when it is considered as an area source. Even with a low percentage of infection in crops, substantial numbers of organisms could be deposited at distances greater than 10 000 m down wind, if an area source is considered, although in most practical circumstances fields would probably be small enough to be regarded as point sources at this distance, and calculations show that very few, if any, bacteria would be deposited.

Despite these reservations, the fact remains that the theoretical numbers of *Erwinia* propagules deposited down wind from the source suggest that airborne transmission could play a role in the spread of the blackleg organism up to a distance of at least 1000 m, according to Gregory's mathematical model. Thus separation distances between crops as presently specified under the official Certification Scheme (only a few metres) are unlikely to be sufficient to avoid re-contamination. However, it is notable that there

are other mathematical models for calculating diffusion and deposition which may give results different from those obtained using Gregory's procedure, but they are more complex to use and require a greater knowledge of meteorological conditions than is generally available, so that they are not considered further in this paper. Nevertheless they should not be discounted, and a useful comparison could be made between Gregory's model and a model based, say, on the generalized atmospheric diffusion equation given by Pasquill (1974). Furthermore, deposition gradients calculated for hypothetical situations would not generally represent the true deposition and infection gradients as they occur in nature, as at any one time only relatively few stems in a crop would be likely to contain the maximum number of viable organisms capable of giving rise to aerosols, and many other factors whose significance is presently unknown, could affect the establishment of infection once deposition has taken place. For instance, the viability of airborne *Erwinia* propagules under different environmental conditions needs to be known before the relation between deposition and infection can be further considered. In this connection, the temperature, the relative humidity of the atmosphere, and the antimicrobial action of open air factor (OAF) must be taken into account (Druett 1973). Investigations on this aspect are now in progress. However, even if viable bacteria reach a potato crop, the likely degree of success in establishing infection is unknown. Deposition on to healthy leaves or stems may not result in infection —presumably the best target site would be damaged areas of stem. On the other hand, bacteria falling on to healthy leaves and stems may be washed down to tubers by rain and establish latent tuber infection. The practical significance of airborne spread of soft rot coliforms is therefore very uncertain, and there is a great deal more to be learned before effective separation distances could be specified with any certainty.

If airborne bacterial aerosols are commonly generated under field conditions, as the foregoing results suggest, it should be possible to catch them from the air down wind of potato crops during rainfall, but so far, very few attempts have been made. In Scotland, small numbers of *E. carotovora* var. *carotovora* have been detected using a high volume Casella sampler (sampling air at 700 l/min) placed 10 m down wind of a potato crop, during a heavy shower. Conditions for aerosol generation were unfavourable because of the generally very dry summer and very low levels of manifest disease in the crop. Previously, Graham & Harrison (1975) reported that airborne soft rot coliform bacteria were caught in Colorado, USA.

Ms Bradley was on an industrial training programme while this work was carried out.

References

ANDERSEN, A. A. 1958 New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology* **76,** 471–484.

BEST, A. C. 1950 The size distribution of raindrops. *Quarterly Journal of the Royal Meteorological Society* **76,** 16–36.

BLANCHARD, D. C. & SYZDEK, L. 1970 Mechanism for the water-to-air transfer and concentration of bacteria. *Science, New York* **170,** 626–628.

CHAMBERLAIN, A. C. 1967 Deposition of particles to natural surfaces. In *Airborne Microbes* 17th Symposium of the Society of General Microbiology, ed. Gregory, P. H. & Monteith, J. L., pp. 138–164, Cambridge: Cambridge University Press.

DARLOW, H. M. 1972 Safety in the microbiological laboratory. In *Safety in Microbiology* ed. Shapton, D. A. & Board, R. G. London: Academic Press.

DRUETT, H. A. 1973 The open air factor. In *Airborne Transmission and Airborne Infection* ed. Hers, J. H. Ph. & Winkler, K. C. pp. 141–149, Utrecht: Oosthoek Publishing Company.

GRAHAM, D. C. & HARDIE, J. L. 1971 Prospects for control of potato blackleg disease by the use of stem cuttings. *Proceedings of the Sixth British Insecticide and Fungicide Conference* **1,** 219–224.

GRAHAM, D. C. & HARRISON, M. D. 1975 Potential spread of *Erwinia* spp. as aerosols. *Phytopathology* **65,** 739–741.

GRAHAM, D. C., QUINN, C. E. & HARRISON, M. D. 1976 Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967–74. *Potato Research* **19,** 3–20.

GREGORY, P. H. 1961 *The Microbiology of the Atmosphere* London: Leonard Hill.

GREGORY, P. H., GUTHRIE, E. J. & BUNCE, M. E. 1959 Experiments on splash dispersal of fungus spores. *Journal of General Microbiology* **20**, 328–354.

HAMBLETON, P. & BENBOUGH, J. E. 1973 Damage to the envelopes of gram-negative bacteria recovered from aerosols. In *Airborne Transmission and Airborne Infection* ed. Hers, J. H. Ph. & Winkler, K. C. pp. 131–134, Utrecht: Oosthoek Publishing Company.

HARRISON, M. D., QUINN, C. E., SELLS, I. A. & GRAHAM, D. C. 1977 Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to re-contamination of pathogen-free potato stocks. *Potato Research* **20,** 37–52.

MAY, K. R. 1967 Physical aspects of sampling airborne microbes. In *Airborne Microbes* 17th Symposium of the Society of General Microbiology, ed. Gregory, P. H. & Monteith, J. L. pp. 60–80, Cambridge: Cambridge University Press.

PASQUILL, F. 1974 Development of practical systems for calculating the concentration pattern from a single source. In *Atmospheric Diffusion* pp. 365–380, Chichester: Ellis Horwood Ltd.

PEROMBELON, M. C. M. & LOWE, R. 1971 The effects of bile salts media and the age of inocula in quantitative studies of populations of *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica*. *Journal of Applied Bacteriology* **34,** 501–506.

STEWART, D. J. 1962 A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. *Nature, London* **195,** 1023.

VENETTE, J. R. & KENNEDY, B. W. 1975 Naturally produced aerosols of *Pseudomonas glycinea*. *Phytopathology* **65,** 737–738.

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Survival of Strains of Soft Rot Coliform Bacteria on Microthreads Exposed in the Laboratory and in the Open Air

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Survival of 5 strains of the plant pathogen, *Erwinia carotovora* var. *atroseptica* and 3 strains of the plant pathogen *E. carotovora* var. *carotovora*, grown in a liquid tryptone medium and held as 'captive' aerosols on gossamer microthreads, was determined under different atmospheric conditions in a controlled environment room and in the open air. Although these bacteria lost viability more quickly than a robust reference strain of *Escherichia coli*, sufficient numbers survived for 15 min or more to indicate that airborne spread of viable propagules could take place, especially under cool humid atmospheric conditions. Cells of one strain of each organism extracted from rotted potato tuber tissue were shown to behave rather like those cultured in the tryptone medium.

THE EPIDEMIOLOGY OF potato blackleg disease, caused by the soft rot coliform bacterium *Erwinia carotovora* var. *atroseptica*, and tuber soft rot, caused by *Erwinia carotovora* var. *carotovora* or *E. carotovora* var. *atroseptica*, has been studied for many years, but is still not fully understood. The introduction of *Erwinia*-free nuclear potato stocks into commerce in 1970 made it easier to observe how or where re-infections of healthy plants occur and thus how soft rot coliform bacteria might spread in the environment (Graham & Hardie 1971; Graham *et al.* 1976; Harrison *et al.* 1977). Graham & Harrison (1975) showed that when simulated raindrops fell on to blackleg-infected potato stems, an aerosol of propagules was generated; this moved readily in slow airstreams and remained viable for more than 1 h in a closed system at 90–95% r.h. and 10·6 °C. They also noted that water drops falling from overhead sprinkler irrigation systems generated an aerosol under field conditions. Venette & Kennedy (1975) showed that an aerosol of *Pseudomonas glycinea* was formed during rainstorms or when overhead sprinklers were used to irrigate soya bean crops affected by bacterial blight. Quantitative studies on aerosol generation, atmospheric diffusion and subsequent deposition made later by Graham *et al.* (1977), indicated that airborne transmission could theoretically be one way that *Erwinia*-free potato plants might be re-contaminated.

As part of the study of the role of airborne transmission in re-contamination it is necessary to know whether the pathogens can survive in an airborne state in small particles and, if so, how long they can remain viable under different atmospheric conditions. This paper describes first, experiments where the survival of several strains of *Erwinia carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora* isolated from

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infected plants, a fly and from the air in the different environments of Scotland and Colorado, were compared with a reference strain of *Escherichia coli* (MRE 162) in a controlled environment room at constant temperature and humidity. This gave information about the relative sensitivity of different strains to exposure in enclosed air. Secondly, survival of one representative strain of each of the 2 organisms was tested in the open air under different weather conditions and at different times of day. In most of the experiments the bacteria were grown in a tryptone-based liquid medium but, because the sensitivity of the cells could be different when grown *in vivo*, comparative tests were done using bacteria extracted from slices of rotting potato tuber. The slices were used as preliminary tests showed that it was often difficult to extract adequate numbers of bacteria from infected stems.

Tests in the controlled environment room and in the open air were done using the microthread technique of May & Druett (1968) for the exposure of 'captive' aerosols. The microthreads were loaded with bacteria using a mobile Henderson apparatus to generate the aerosol (Druett 1969). This method, where the organisms are held on ultra-fine threads of spider gossamer wound on metal frames, has been shown to give results quantitatively similar to those found for true airborne particles and is commonly used to assess bacterial survival in the open air (Southey & Harper 1971; Hugh-Jones *et al.* 1973).

Materials and Methods

Bacterial strains

Details of the strains of *Erwinia* used in the experiments are summarized in Table 1. The strain of *E. carotovora* var. *carotovora* isolated from a fly was probably originally derived from potato material as the insect was caught at a waste potato dump (Harrison *et al.* 1977). The strain of *E. carotovora* var. *atroseptica* obtained from the air was caught with an Andersen sampler down wind of a potato crop affected by blackleg disease.

The reference organism *Esch. coli* MRE 162 is commonly used in experiments on atmospheric survival of bacteria on microthreads and is considered to be an aerosol-robust organism (Southey & Harper 1971).

TABLE 1

Strains of Erwinia *used in the experiments*

Strain		Source	Place of origin
E. carotovora var. *atroseptica*	G125	Potato tuber	Scotland
	G331	Potato tuber	Scotland
	G336	Potato stem	Scotland
	F24/1	Fly	Scotland
	J10a	Potato stem	Colorado, U.S.A.
	AS12	Air	Colorado, U.S.A.
E. carotovora var. *carotovora*	G123	Potato tuber	Scotland
	G324	Swede turnip	Scotland
	H_3A_5	Potato tuber	Colorado, U.S.A.

Suspensions

Soft rot coliform bacteria

Stock cultures were maintained on nutrient agar slopes (g/l: peptone (Oxoid), 10; Lab–Lemco powder (Oxoid), 5, and agar, 20). Batches of suspension were prepared by dispersing a heaped loopful (1 mm diam. loop) from a 24 h slope culture into 10 ml of nutrient broth. After 24 h incubation at 26 °C, four 50 ml volumes of tryptone medium (Anderson 1966) in flasks (500 ml) were each inoculated with 0·5 ml of the broth culture. In later experiments, each of two 500 ml flasks containing 100 ml of tryptone medium was inoculated with 1 ml of the broth culture; either method gave roughly the same yield of organisms. After incubation in a water bath fitted with a reciprocal shaker (90 oscillations of 1·5 cm amplitude/min) for 48 h at 26 °C, the tryptone medium was centrifuged (1200 **g** for 15 min) and the bacterial pellet resuspended in 12 ml of supernatant. The viable cell counts from cultures prepared in this way ranged from 2·6–6·3 × 10^{10}/ml. Suspensions were stored for 48 h at 4 °C before use to enable counts to be completed; this did not affect viability of the organisms in the suspension.

For experiments with bacteria grown in potato tuber tissue, 35 tuber slices (*ca.* 1 cm in thickness) were each inoculated with a large loopful of a 24–48 h old nutrient agar culture, and incubated for 24 h at 26 °C on damp filter paper in Petri dishes. Rotted tissue was suspended in *ca.* 20 ml sterile water in large test tubes and mixed for 0·5 min using a Fisons 'Whirlimixer'. The suspension was filtered through muslin and the fluid centrifuged (200 **g** for 5 min) to remove plant cell debris. The supernatant was decanted and centrifuged (2500 **g** for 15 min) to recover the bacteria and the pellet resuspended in 15 ml sterile water. The viable cell counts varied from 5·6–7·3 × 10^{10}/ml. Suspensions were stored at 4 °C for 48 h before use.

Escherichia coli *MRE 162*

The stock culture was maintained on nutrient agar slopes. Suspensions were prepared in the same way as for the soft rot coliforms, but three 500 ml flasks each containing 100 ml of tryptone medium were inoculated and incubated for 24 h at 37 °C. Counts of viable cells varied from 2·7–4·3 × 10^{10}/ml.

Bacillus subtilis *var.* niger spores (B. globigii, BG)

A single batch of spores received from the Microbiological Research Establishment (MRE) was used as a tracer organism in all viability tests (Anderson & Cox 1967). It was stored at 4 °C and, immediately before use in each experiment, a small volume of spore suspension was heated to 60 °C for 1 h in a water bath to destroy any partly germinated spores or vegetative cells that might have been present.

Preparation of suspensions for aerosol generation

Cells grown in liquid media were sprayed from a whole culture diluted with an equal volume of water containing BG spores (50% mother liquor). Cells extracted from potato tuber tissue were sprayed from distilled water plus BG spores. Test cell concentrations were adjusted to approx. 4·0 × 10^{10}/ml and sufficient BG suspension added to give a ratio of test cells to BG of approx. 3 : 1.

Apparatus for generating aerosols, loading microthreads, exposing organisms and sampling clouds

Generation of aerosol

Aerosols were generated with a modified Collison atomizer (Druett 1969) attached to the mobile Henderson apparatus.

Loading and exposing microthreads

Microthreads were loaded with bacteria by drawing the aerosol from the mixer tube of the Henderson apparatus, through a set of 20 frames aligned in an airtight tube [referred to as a 'sow' by May & Druett (1968)] for 1 min. All loading was done in the sows at an r.h. of 90–95% to simulate the conditions under which aerosols are generated in nature; this r.h. would be achieved in the immediate environment when rain or water drops from overhead sprinklers fall on infected plant material. The microthread frames were exposed to the air under circular metal canopies [referred to as 'roundabouts' (Druett & May 1969)], which allowed full air circulation but gave some physical protection to the threads. In the open air, the roundabouts were protected from direct sunlight or rain by an open-sided shelter. Groups of 3 frames were extracted for viable cell assessment at 0, 5, 15, 30 min and 1 h, and 2 frames were removed 2 h after starting exposure. Three frames remained in the sow and were usually extracted after 2 h (although a few were done at 1 h) to compare viability of organisms held in the atmosphere of the sow with those exposed to outside air. Ten minutes elapsed between loading microthreads and the beginning of the exposure. To ensure that very little outside air entered the sows, they were sealed as quickly as possible after removal of frames. The Henderson apparatus was operated in a room where the ambient temperature could be adjusted to correspond with that of the air to which the microthreads were exposed. The proportion of viable organisms was assessed by the BG tracer method as described below.

Cloud sampling from the Henderson apparatus

Samples were collected from the mixer tube for 1 min at the beginning and end of each experiment with Porton raised impingers (May & Harper 1957) at a flow rate of 11 l/min. The collecting liquid was the same as the diluent used for viable assessment (see below).

Controlled environment room and environmental conditions

The room was part of a large air-conditioned installation which was capable of maintaining constant air temperatures and humidities over a range 10–25 $\pm$ 1 °C and 50–100 $\pm$ 5% r.h. In the experiments in the controlled environment room an arbitrary temperature of 20 °C and relative humidity of 65% was chosen to expose the test cells to some degree of stress, and thus to give an indication of the relative capacity for survival of different isolates of soft rot coliform bacteria under these conditions.

Method of viable assessments of test bacteria

Viable assessment

Suspension, cloud and microthread samples were diluted in phosphate buffer alginate (Anderson 1966), containing 1 ml of a 10% (w/v) solution of GEC Silicone 60 Antifoam/l. Volumes (0·25 ml) for each of the dilutions assessed were plated on to the

surfaces of each of 4 Petri dishes containing tryptone glucose agar previously dried for 3 h/37 °C. All Petri dishes used for the soft rot coliform bacteria experiments were incubated at 26 °C for 48 h and the colonies then counted. For *Esch. coli* counts the Petri dishes were incubated at 37 °C for 24 h. The tryptone glucose agar medium consisted of (g/l): Tryptone (Oxoid), 10; NaCl, 5, and agar, 15 (pH 7·0). After sterilization, 20 ml of a 50% (w/v) filter-sterilized solution of glucose was added (Dark, pers. comm.).

Determination of the viability of test bacteria

The concentration of viable cells in an aerosol is reduced by physical loss as well as decay of viability, and it is therefore necessary to distinguish between these processes. The procedure adopted was to mix the test bacterial cells with a tracer organism, BG, which was subject to the same physical loss, but which was known to suffer relatively little viable decay under the test conditions (Southey & Harper 1971). The ratio of viable test cells to BG in the suspensions was equated to 100% viability and the ratios found in the cloud and microthread samples were then expressed as percentages in terms of this ratio. The viability of organisms in the cloud samples was determined to check that the apparatus was working correctly and that the cells were not affected adversely by the aerosolization process.

Results

Survival of strains of soft rot coliform bacteria in the controlled environment room at 20 °C and 65% r.h.

Results for *E. carotovora* var. *atroseptica* G125, G336 and F24/1, *E. carotovora* var. *carotovora* H_3A_5, and *Esch. coli* MRE 162 are illustrated graphically in Fig. 1. Tests showed that *E. carotovora* var. *atroseptica* J10a, G331 and AS12, and *E. carotovora* var. *carotovora* G123 behaved very like strains G125 and G336, whereas *E. carotovora* var. *carotovora* G324 was very similar to *E. carotovora* var. *carotovora* H_3A_5 and *E. carotovora* var. *atroseptica* F24/1; the data for J10a, G331, AS12 and G324 could not be included in Fig. 1 as the graphs would almost superimpose on one another.

Thus the *Erwinia* strains could be roughly divided into 2 groups. One group, to which most strains of *E. carotovora* var. *atroseptica* belonged, lost viability comparatively quickly. In most cases no viable organisms could be detected after 2 h, and in every experiment, survival was below 20% after 5 min, 10% after 15 min and 2% after 30 min exposure. Survival was better in the sow, presumably because the organisms did not suffer the trauma of exposure to air of lower humidity, but even so, survival had fallen to around 2% or less after 2 h. The other group, made up of one strain of *E. carotovora* var. *atroseptica* and 2 of *E. carotovora* var. *carotovora*, lost viability more slowly. With the exception of *E. carotovora* var. *atroseptica* F24/1 where viability was only 10% after 5 min exposure, there was more than 25% survival at 5 min, 5% at 1 h, and 2% after 2 h exposure. There was also noticeably better survival in the sow, varying from 6% to 12·8% after 2 h. Neither group of organisms tolerated the conditions as well as the reference strain, *Esch. coli* MRE 162, which showed a survival of more than 30% after 2 h both in the room and in the sow. This is perhaps not surprising as strain MRE 162 is known to be a robust organism, (Southey & Harper 1971) and to withstand exposure well.

Results with the cells extracted from potato tubers were generally similar to those for the same organisms grown in liquid media. As before data are not included in Fig. 1 as the curves would almost superimpose on those illustrated. With *E. carotovora* var. *atroseptica* G125, survival was just below 10% after 5 min, 1% at 1 h and no viable organisms were found after 2 h exposure. With *E. carotovora* var. *carotovora* G324 there was approx. 1% survival after 2 h, and 5% at 1 h although only *ca.* 6% after 5

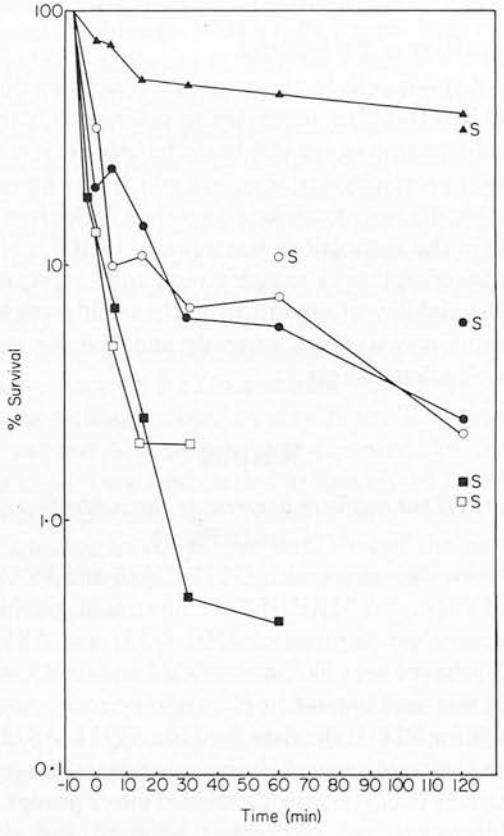

Fig. 1. The survival of *Erwinia carotovora* var. *atroseptica* G125 (□) G336 (■) F24/1 (○), *Erwinia carotovora* var. *carotovora* H_3A_5 (●) and *Escherichia coli* MRE 162 (▲) exposed on microthreads to air in controlled environment room at 20 °C and 65% r.h. Sow (S).

min. Only 2 experiments were done, however, and experience with the method indicates that erratic results, such as that for the 5 min exposure, are encountered from time to time. Moreover, another factor affecting viability may have been that these cells were sprayed from distilled water, and not from 50% mother liquor. It is known that the composition of the suspending medium affects survival in the air and that substances in mother liquor can have a protectant effect (Anderson & Cox 1967).

Survival of Erwinia carotovora *var.* atroseptica *G125 and* Erwinia carotovora *var.* carotovora *G324 in the open air*

The isolates G125 and G324 were chosen for study in the open air as they were representative of the 2 groups distinguished by exposure in the controlled environment

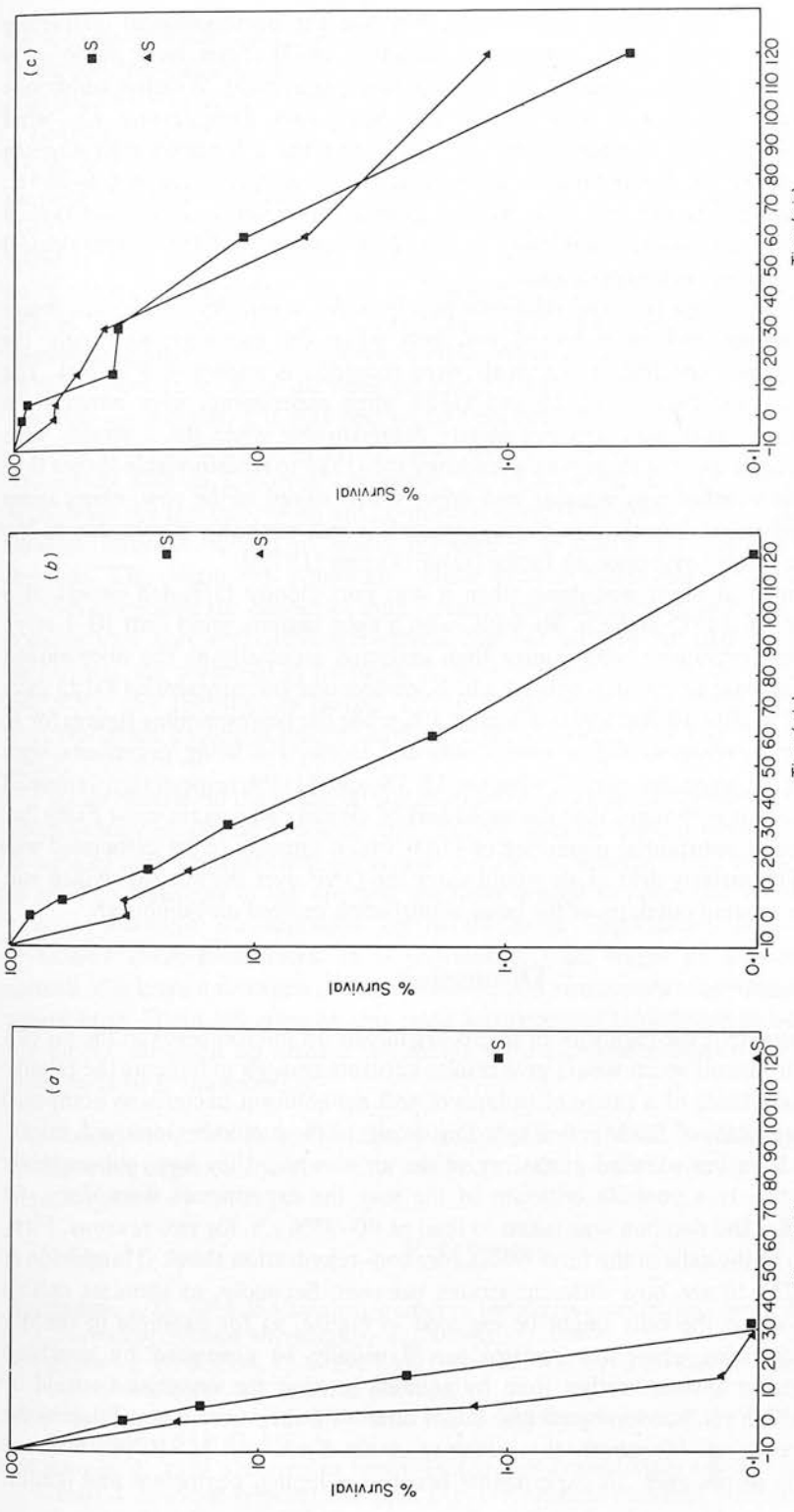

Fig. 2. The survival of *Erwinia carotovora* var. *atroseptica* G125 (▲) and *Erwinia carotovora* var. *carotovora* G324 (■) exposed on microthreads to the open air. (*a*) Morning, 0/8 cloud cover. Temperature 18–26 °C; r.h. 43–70%. Wind: direction 230–310°, and speed 0–1·5 m/sec. (*b*) Morning, 4/8–7/8 cloud cover. Temperature 14·5–16·5 °C; r.h. 71–79%. Wind: direction 40–90°, and speed 0·5–2·2 m/sec. (*c*) Evening, 8/8 cloud cover. Temperature 12–12·5 °C; r.h. 86–90% (rain falling). Wind: direction 225°, and speed 0·9–2·1 m/sec. Sow (S).

room. Moreover, it was already known that they had the bacteriological characters typical of the 2 varieties of *E. carotovora* (Graham 1972). Tests were made on 6 occasions, 3 during the day, 2 during the evening, and one at night. Weather conditions varied from warm sun, to cool dark conditions in heavy rain. Temperature, r.h., wind speed and direction were recorded every 15 min during the 2 h period microthreads were exposed. Over the 6 experiments, these ranged from temperatures of 6·6–26 °C, 43–94% r.h. and windspeed 0–5 m/s. Results from 3 experiments, illustrating typical levels of survival are shown graphically in Fig. 2; a summary of the meteorological conditions is given for each experiment.

In general the bacteria survived relatively poorly under warm dry conditions, better when it was cooler and more humid and best when the humidity was high: the experiment in which the highest survivals were recorded is shown in Fig 2(*c*). The difference in survival between G125 and G324 when experiments were done in the controlled environment room was not clearly demonstrable when the 2 strains were exposed in the open air, but there was a tendency for G324 to remain viable longer than G125 when the weather was warmer and drier. When stored in the sow, many more organisms survived at 2 h than in the open air; this was probably partly due to the presence of the highly toxic open air factor [OAF; Druett (1973)].

The experiment at night was done when it was part cloudy (3/8–4/8 cloud), at a temperature of 13–14 °C and r.h. 90–94%, with a light easterly wind drift (0–1 m/s). Viability of both organisms was poorer than expected especially as the microthread loading r.h. was close to the atmospheric r.h. *E. carotovora* var. *atroseptica* G125 gave survivals of 5·1% after 30 min and 0·3% after 1 h, while the corresponding figures for *E. carotovora* var. *carotovora* G324 were 4·4% and 0·2%. No living organisms were found after the 2 h exposure period, whereas 13·5% and 51·1% respectively, remained viable in the sow. It is thought that the rapid loss of viability was again most likely due to the presence of substantial quantities of OAF which appears to be associated with urban areas. The easterly drift of air would carry the OAF over the test site, which was situated on the western outskirts of the large conurbation centred on Edinburgh.

Discussion

It was considered that the exposure of micro-organisms on microthreads in the air of a controlled environment room would give results accurate enough to indicate the relative survival characteristics of a range of isolates of soft rot coliform bacteria as compared with a reference strain of *Esch. coli*. Regarding details of the methods employed, microthreads could have been loaded at the r.h. of the air to which they were subsequently exposed, and this is a possible criticism of the way the experiments were done. As mentioned earlier the decision was taken to load at 90–95% r.h. for two reasons. First, to apply stress to the cells in the form of dehydration–rehydration shock (Hambleton & Benbough 1973), to see how different strains behaved. Secondly, to simulate certain conditions to which the cells might be exposed in nature, as for example in the dry climate of Colorado, where the aerosol would usually be generated by overhead sprinkler irrigation systems rather than by rainfall, so that the organisms would be aerosolized at high r.h. but soon pass into much drier air if they were formed during the day or early evening. Moreover, the reference strain *Esch. coli* MRE 162 was not included in any of the open air experiments because of limited equipment and labour.

Lack of comparative data does not, however, invalidate the information on the survival of the soft rot coliform bacteria themselves, although it is possible that the air at the site contained varying concentrations of OAF as the laboratory is close to a city.

Bearing these reservations in mind, the experiments show that laboratory grown strains of *E. carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora*, when held on microthreads to simulate the airborne state, can survive for an appreciable time at 20 °C and 65% r.h. in an air-conditioned atmosphere, although not as long as a robust strain of *Esch. coli*. Organisms found in the moist cool climate of Scotland and in the more extreme continental climate of Colorado behaved in the same way. Moreover, cells of Scottish isolates of one strain of each of these organisms grown *in vivo* reacted somewhat similarly to those grown *in vitro*. When cells of these same 2 strains, grown *in vitro*, were exposed in the open air under a variety of weather conditions, survival was also appreciable, but was best under cool conditions at high humidity. These tests indicate that there must be many times during summer and autumn in Britain and sometimes also in Colorado, especially at night when cool temperatures and high humidities are the rule, when environmental conditions allow survival long enough for possible airborne spread of viable propagules to occur from crop to crop over a distance. The warm dry conditions which exist in Colorado during the day would probably preclude the danger of long distance dissemination of aerosols. It is, however, notable that soft rot coliform bacteria did not survive so well as a strain of *E. amylovora*, the pathogen of fireblight disease of pome-fruits, which behaved like *Esch. coli* MRE 162 (Southey & Harper 1971); *E. amylovora* appears to be the only other bacterial plant pathogen whose survival in the airborne state has been investigated in detail.

Further studies on the potential significance of airborne transmission in relation to the epidemiology of potato blackleg and soft rot diseases should include a study of the process of aerosol generation under field conditions, determination of the concentration of viable propagules in the air, and the dose needed to infect potato plants when the airborne bacteria are deposited on to the crop. Experiments are in progress to investigate these parameters. In a preliminary trial, where an artificially generated aerosol of a known serotype of *E. carotovora* var. *carotovora* was released upwind of a potato crop, latent infection by this same serotype was later found to be established in the tubers, although no disease symptoms were manifest either on the above ground plant parts or in the tubers.

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References

ANDERSON, J. D. 1966 Biochemical studies of lethal processes in aerosols of *Escherichia coli*. *Journal of General Microbiology* **45**, 303–313.

ANDERSON, J. D. & COX, C. S. 1967 Microbial survival. Symposia of the Society for General Microbiology No. 17, *Airborne Microbes*.

DRUETT, H. A. 1969 A mobile form of the Henderson apparatus. *Journal of Hygiene, Cambridge* **67**, 437–448.

DRUETT, H. A. 1973 The open air factor. In *Airborne Transmission and Airborne Infection* ed. Hers, J. H. Ph. & Winkler, K. C., pp. 141–149. Utrecht: Oosthoek Publishing Company.

DRUETT, H. A. & MAY, K. R. 1969 The open air factor. *New Scientist* 13 March, 579–581.

GRAHAM, D. C. 1972 Identification of soft rot coliform bacteria. *Proceedings of the Third International Conference of Plant Pathogenic Bacteria* (*Wageningen*, 1971). Pudoc, Wageningen, pp. 273–279.

GRAHAM, D. C. & HARDIE, J. L. 1971 Prospects for control of potato blackleg disease by the use of stem cuttings. *Proceedings of the Sixth British Insecticide and Fungicide Conference* **1,** 219–224.

GRAHAM, D. C. & HARRISON, M. D. 1975 Potential spread of *Erwinia* spp. as aerosols. *Phytopathology* **65,** 739–741.

GRAHAM, D. C., QUINN, C. E. & HARRISON, M. D. 1976 Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967–74. *Potato Research* **19,** 3–20.

GRAHAM, D. C., QUINN, C. E. & BRADLEY, L. F. 1977 Quantitative studies on the generation of aerosols of *Erwinia carotovora* var. *atroseptica* by simulated raindrop impaction on blackleg-infected potato stems. *Journal of Applied Bacteriology* **43,** 413–424.

HAMBLETON, P. & BENBOUGH, J. E. 1973 Damage to the envelopes of Gram-negative bacteria recovered from aerosols. In *Airborne Transmission and Airborne Infection* ed. Hers, J. H. Ph. & Winkler, K. C., pp. 131–134. Utrecht: Oosthoek Publishing Company.

HARRISON, M. D., QUINN, C. E., SELLS, I. A. & GRAHAM, D. C. 1977 Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to re-contamination of pathogen-free potato stocks. *Potato Research* **20,** 37–52.

HUGH-JONES, M., ALLAN, W. H., DARK, F. A. & HARPER, G. J. 1973 The evidence for the airborne spread of Newcastle disease. *Journal of Hygiene, Cambridge* **71,** 325–339.

MAY, K. R. & DRUETT, H. A. 1968 A microthread technique for studying the viability of microbes in a simulated airborne state. *Journal of General Microbiology* **51,** 353–366.

MAY, K. R. & HARPER, G. J. 1957 The efficiency of various liquid impinger samplers in bacterial aerosols. *British Journal of Industrial Medicine* **14,** 287–297.

SOUTHEY, R. F. W. & HARPER, G. J. 1971 The survival of *Erwinia amylovora* in airborne particles: tests in the laboratory and in the open air. *Journal of Applied Bacteriology* **34,** 547–556.

VENETTE, J. R. & KENNEDY, B. W. 1975 Naturally produced aerosols of *Pseudomonas glycinea*. *Phytopathology* **65,** 737–738.

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Soft Rot *Erwinia* Bacteria in the Atmospheric Bacterial Aerosol

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Using a Casella High Volume Airborne Bacteria Sampler, the soft rot coliform bacteria *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were caught from the open air during rainfall in mid to late summer, autumn and early winter, but not in late winter, spring or early summer. They were not found every time it rained, and never when the weather was dry. The bacteria were caught close to potato crops, but there were several occasions when they were found at sites where there were no such crops or at times of year after potato crops had been harvested. The sources of the organisms are uncertain, but those obtained close to potato crops may have originated there. The results are considered to provide further evidence that airborne spread and subsequent deposition of viable bacteria could cause contamination of *Erwinia*-free potato stocks, and suggest that rainfall is a major generator of the general atmospheric bacterial aerosol.

IN CONTINUING STUDIES on the epidemiology of potato blackleg disease, Graham & Harrison (1975) showed that when simulated raindrops fell on to potato stems infected with the potato blackleg organism, *Erwinia carotovora* var. *atroseptica*, an aerosol of viable bacteria was readily generated in a specially designed raindrop simulator/wind tunnel. Later Graham *et al.* (1977) modified the apparatus so that quantitative studies could be made on aerosol generation, atmospheric diffusion and subsequent aerosol deposition. The purpose of these investigations was to try to discover if atmospheric spread of soft rot bacteria could occur and therefore that *Erwinia*-free potato stocks might be re-contaminated by airborne organisms. The results strongly suggested that an atmospheric aerosol might be generated naturally when raindrops or water drops from overhead irrigation sprinklers fell on to infected blackleg stems in the field. Moreover, using the 'captive' aerosol method (May & Druett 1968), Graham *et al.* (1979) demonstrated that viable cells of both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* could survive in small particles in the open air long enough for them to spread from crop to crop over a distance, especially in cool moist conditions.

The object of the present experiments was to attempt to catch airborne *Erwinia* propagules down wind close to potato crops when it was raining and when it was dry, at various sites at different times of year and over a two-year period. Investigations were confined to the Edinburgh area, partly because of ease of access, partly because potato crops are commonly grown to the east of the city (in East Lothian), and partly because the area to the southwest of the city included Ingraston Farm (Site 5 in Fig. 1) situated in a region where very few commercial potato crops are grown. On this farm the Agricultural Scientific Services of the Department of Agriculture and Fisheries produce the 'nuclear' stock of *Erwinia*-free potatoes using tested potato stem cuttings, from which crops of the 'Virus-Tested Stem Cutting' (VTSC) grade—the highest grade

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of certified seed potatoes produced in Scotland—are propagated. Re-contamination of VTSC material with *Erwinia* spp. and other pathogens has been closely monitored since *Erwinia*-free cuttings were first produced in quantity in 1967, and tuber stocks released to specialist commercial VTSC raisers in 1970 (Graham & Hardie 1971; Graham *et al.* 1976). Air sampling was seen as another step towards understanding as much as possible about the occurrence of potato pathogens in the Ingraston environment.

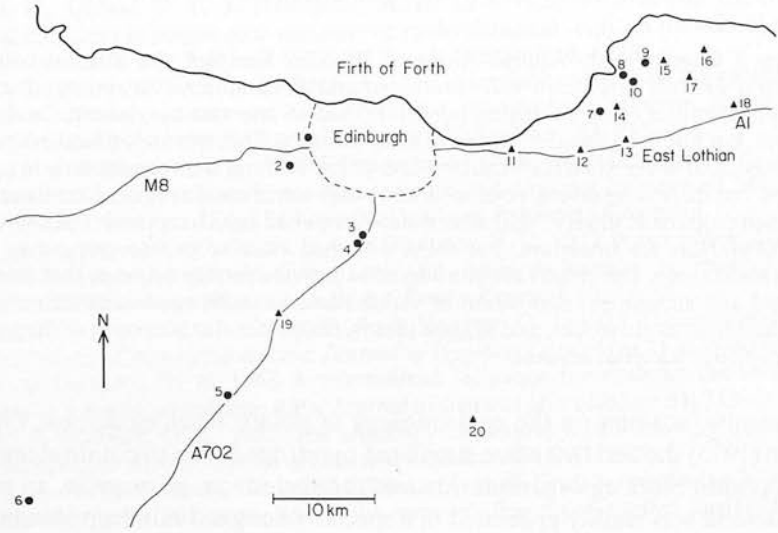

Fig. 1. Map showing the location of the various sample sites. Soft rot coliform bacteria caught ●, not caught ▲. A1, A702 and M8—main roads.

If soft rot coliforms were easily caught close to potato crops, attempts would then be made to catch them at places in arable areas where no potatoes could be seen in the vicinity, at times of year when potato crops were not growing, and in non-arable areas.

Before this study, very little was known about the presence or absence of soft rot coliforms in the open air. The only previously recorded cases were by Graham & Harrison (1975), where an un-named soft rot coliform was caught with an Andersen Sampler (Andersen 1958) down wind of a potato crop when overhead sprinklers were in use in Colorado, U.S.A., and by Graham *et al.* (1977) in Scotland, where *E. carotovora* var. *carotovora* was caught down wind of a potato crop during a heavy rain shower, using a Casella High Volume Bacteria Sampler (C. F. Casella and Co. Ltd., London). Perombelon (1977) recorded formation of airborne *Erwinia* by haulm pulverization.

Materials and Methods

Air samplers and sampling

Preliminary tests comparing the Casella Sampler with an MRE Cyclone Sampler (based on the device designed by Errington & Powell 1969) showed that the Casella

apparatus was better than the latter for catching an artificially generated aerosol of *E. carotovora* var. *atroseptica* which was allowed to drift down wind towards the samplers at distances varying up to about 400 m. The Casella Sampler, sampling at the rate of 700 l/min was therefore used in all field experiments.

The sampler was mounted at the rear of a Landrover vehicle at a height of 1·3 m above ground level. Its vacuum pump was supplied with electricity from a mobile generator. The rear of the vehicle was always pointed in the direction of the wind, thus acting as a baffle, probably achieving air flow conditions roughly equivalent to stagnation point sampling (May 1967). The sampler was always run for a period of 5 min per plate. Different numbers of plates were run at each sampling for various practical reasons but mainly because of the differing periods of rainfall.

Sample sites and meteorological conditions

The various sites are indicated on the map (Fig. 1). The majority were in part of the county of East Lothian, where large areas of potatoes and brassicas are cultivated. The rest were widespread, ranging from East Craigs (Site 1) and Gogarbank (Site 2) on the western outskirts of Edinburgh, to the Lochlyoch area (Site 6) some 50 km southwest of the city.

The meterological conditions were recorded during sampling, including presence or absence of rain (a subjective impression of the heaviness of the rainfall was recorded in three categories, light, moderate and heavy), temperature, r.h. and wind speed and direction. Some experiments were done during the daytime, some at dusk and some at night.

Media and identification of bacteria

Bacteria were deposited on to MacConkey pectate medium (Stewart 1962), containing 200 mg/kg cycloheximide (Actidione, Upjohn) to limit growth of fungi. The medium was dispensed into 140 mm diam. Petri dishes, which were incubated for 48–72 h at 26°C and colonies removed to Nutrient Agar slopes (Oxoid) for identification. The bacteria were purified and subsequently identified by the methods of Dye (1969) and Graham (1972) as described by Graham *et al.* (1976).

Results

The experiments extended over the period August 1977 to October 1979. The results are summarized in Table 1.

In 11 of 28 experiments in which soft rot coliforms were detected, the sampler was placed within 10–20 m down wind of blackleg-infected potato crops. The rest of the samples were taken at sites where no potato crops were seen in the vicinity, or at times of year when crops had been harvested, and the haulm was dead.

These results show that soft rot coliform bacteria, *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica*, can be caught easily from the open air during rainfall at different places near Edinburgh and at other sites, especially in East Lothian, but never when it was dry. Sometimes only one or other of the two organisms was found, whereas on other occasions both were caught. The bacteria appeared to be present

TABLE 1

Results of sampling air for the presence of soft rot coliform bacteria at sites near and around Edinburgh at different times of year

Date of sampling	Site number	Potato crop location	Category of rain	Temperature (°C): r.h. (%)	Wind direction and speed (m/s)	Number of plates exposed	Total number of *Erwinia* colonies	Number of colonies identified	Number of *Erwinia carotovora* var. *carotovora*	Number of *Erwinia carotovora* var. *atroseptica*
31.8.77	3	Within 10 m	Light	14·5:80	*W 1·5	2	1	1	0	1
6.9.77	3	Within 10 m	Moderate	15·0:98	W 9·0	8	22	21	2	19
8.9.77	3	Within 10 m	Moderate	8·.5:90	W 2·5	10	3	3	0	3
27.9.77	9	None nearby	Heavy	11·5:98	E 7·5	3	>100	22	22	0
27.9.77	10	None nearby	Moderate	11·5:98	E 6·5	3	11	8	7	1
7.10.77	4	None nearby	Heavy	10·0:100	E 2·5	3	6	6	4	2
24.10.77	3	Within 20 m	Light	12·5:96	W 9·0	2	1	1	0	1
30.10.77	1	None nearby	Light	14·7:85	SW 7·0	8	1	1	0	1
30.10.77	2	None nearby	Heavy	14·0:92	SW 5·3	14	1	1	0	1
3.11.77	7	Crops lifted	Moderate	7·5:85	W 7·5	7	1	1	1	0
11.11.77	5	Crops lifted	Moderate	7·5:90	W 8·5	9	1	1	1	0
23.12.77	9	Crops lifted	Moderate	9·0:91	E 1·8	4	11	11	3	8
4.7.78	8	Within 10 m	Moderate	11·3:98	N 1·0	3	7	7	4	3
1.8.78	8	Within 10 m	Heavy	13·5:95	E 2·4	4	7	7	3	4
5.9.78	2	Within 10 m	Moderate	12·0:94	E 1·3	5	2	2	2	0
7.9.78	2	Within 20 m	Light	17·0:88	W 3·3	3	39	16	0	16
8.9.78	1	None nearby	Heavy	15·5:100	W 3·1	10	>125	37	0	37
28.9.78	6	None nearby	Moderate	10·0:94	SW 3·0	8	33	17	5	12
24.10.78	1	None nearby	Light	11·0:88	W 3·7	3	3	3	3	0
25.10.78	10	Within 10 m	Light	10·0:100	SW 0·2	3	2	2	1	1
31.10.78	1	Crops lifted	Light	10·5:94	SW 1·3	14	31	28	25	3
13.11.78	6	Crops lifted	Heavy	4·5:100	W 4·3	6	3	3	1	2
25.7.79	8	Within 20 m	Heavy	16·5:90	W 1·5	5	>100	15	3	12
7.8.79	1	None nearby	Moderate	12·0:98	W 0·7	6	1	1	0	1
20·8·79	6	Within 10 m	Moderate	10·0:94	SW 6·0	4	18	18	15	3
20.9.79	1	None nearby	Heavy	8·5:93	SW 3·5	7	3	3	0	3
25.9.79	6	None nearby	Heavy	14·0:95	SW 6·0	5	>175	26	2	24
11.10.79	1	None nearby	Light	13·0:87	S 3·8	2	1	1	1	0

The following lists the dates, sites and presence or absence of rain (light, L; moderate, M; heavy, H) when no soft rot coliform bacteria were caught. 27.9.77: 13, rain L; 17, rain L; 15, rain L. 6.10.77: 5, dry then rain L. 7.10.77: 5, rain L. 21.10.77: 1, dry. 23.10.77: 1, rain L; 2, rain L. 24.10.77: 4, rain L; 19, rain L. 3.11.77: 16, dry. 7.11.77: 11, rain L; 12, rain L; 13, rain L; 18, dry then rain L; 17, rain M; 14, dry. 10.11.77: 2, rain L. 23.11.77: 1, rain L; 3, rain L; 4, rain L; 5, rain M; 19, rain L; 7, rain M. 8.12.77: 12, rain L. 12.12.77: 14, dry. 23.12.77: 10, rain L; 14, rain L. 6.2.78: 9, rain M; 10, rain L; 15, rain L; 8, rain L; 14, rain L. 1.3.78: 9, rain L. 13.3.78: 14, rain M; 9, rain L; 8, rain L. 22.3.78: 14, rain H; 9, rain L; 3, rain L; 5, rain M. 28.3.78: 5, rain L. 27.4.78: 9, rain M; 8, rain M; 10, rain M; 7, rain M. 22.5.78: 9, rain L; 10, rain L; 14, rain L; 11, rain M. 8.6.78: 5, rain M then dry. 20.6.78: 9, dry; 2, rain M. 21.6.78: 5, rain M; 4, rain H. 22.6.78: 9, dry then rain M; 14, rain L. 3.7.78: 5, rain H. 4.7.78: 9, rain L; 10, rain M. 20.7.78: 2, rain L then dry. 25.7.78: 1, dry. 26.7.78: 8, rain L; 9, rain L; 14, rain M; 20, rain L then dry. 1.8.78: 8, dry; 9, dry then rain L; 17, dry; 15, rain M; 10, rain L. 14; rain L. 3.8.78: 5, rain L. 7.8.78: 2, rain L. 14.8.78: 1, dry then rain L. 21.8.78: 2, rain L. 31.8.78: 3, rain L. 7.9.78: 5, rain L. 20.9.78: 2, dry. 18.10.78: 6, dry. 25.10.78: 8, rain L. 10.11.78: 6, rain L. 13.11.78: 1, rain H. 8.12.78: 1, rain L. 15.12.78: 1, rain M then dry. 16.1.79: 1, rain L. 18.1.79: 1, rain L. 6.3.79: 1, dry then rain M. 26.4.79: 1, dry. 15.5.79: 6, dry. 16.5.79: 2, rain L. 23.5.79: 6, rain L. 28.5.79: 6, dry. 29.5.79: 1, dry. 16.7.79: 1, rain H. 17.7.79: 6, rain L. 6.8.79: 6, dry.

regularly in the air during mid to late summer to early winter. In this connection, it is notable that the summers and autumns of 1977, 1978 and 1979 were wet and therefore favourable for multiplication of the organisms in the host plant and consequent aerosol generation; presumably drier periods would be less favourable and soft rot coliforms more difficult to find. In some experiments what seemed to us to be surprisingly large numbers of both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* grew on the plates, indicating that they might make up a considerable proportion of the natural atmospheric bacterial aerosol, especially in autumn and early winter. By contrast, the organisms were absent from the air from late winter into early summer.

Discussion

These observations strengthen the view that naturally occurring viable airborne soft rot coliforms could be deposited on, and might contaminate, *Erwinia*-free VTSC stocks of potatoes, and could help to explain why blackleg disease and soft rot infections can appear in VTSC material in early stages of multiplication despite the adoption of strict hygienic measures by raisers. Other methods of spread cannot, of course, be discounted, for example, transmission by insects (Harrison *et al.* 1977) and by machinery (Graham & Hardie 1971). Additionally, Perombelon (1977) has shown that soft rot coliform aerosols can be generated by mechanical destruction of potato haulm.

As indicated by Graham *et al.* (1979), an artificially generated aerosol of a known arbitrary serotype of *E. carotovora* var. *carotovora* (Graham *et al.* 1976) released up wind of a VTSC potato crop was later recovered from the tubers. This experiment has now been repeated, with the same result. These observations therefore suggest it should be possible to find soft rot coliforms associated with tubers raised directly from tested cuttings and grown in the open air, even when other potato stocks are not close by, because both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were commonly airborne during the potato growing seasons in 1977, 1978 and 1979. Experiments to study this in depth are in progress, but we have already isolated *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* from tubers produced from stem cuttings and grown in the open at East Craigs—a site where *Erwinia* spp. were often caught from the air, but where no potato crops were growing nearby. As mentioned above it is possible that the bacteria reached the cuttings in other ways, and many more experiments are necessary before the practical significance of airborne spread in re-contamination of VTSC potato stocks can be assessed. An important factor still to be quantified is the number of bacteria which have to be deposited on the above ground parts of potato plants to establish infection.

The origin of airborne soft rot coliforms is uncertain although presumably those caught close to potato crops came mostly from the crops themselves. Further work is needed to learn more about the times when these bacteria occur in the open air, the effect of weather conditions, and their numbers in the air. Moreover, experiments need to be done by sampling air up wind and down wind of crops simultaneously, to find out if the bacteria caught down wind originate from the crops. The presence of *Erwinia* spp. in the air long after potato harvest and death of haulm, suggests that there are sources other than potatoes. In East Lothian at least, turnips and other brassica crops

might provide the sources, but so far this possibility has not been investigated in detail.

It is notable that no soft rot coliform bacteria were isolated from the air on some occasions when it rained, and never in dry weather, although a few were caught when it was dry overhead but frontal rain or showers could be seen falling nearby. Time of day, temperature, r.h. and other meteorological factors except rainfall did not appear to influence the occurrence of soft rot bacteria in the air, at least over the range of conditions shown in Table 1. This was not unexpected in view of the results of airborne particle survival tests described by Graham *et al.* (1979).

There was evidence (which will be described elsewhere) that the heavier it rained, the more bacteria of various kinds appeared on the plates (although MacConkey pectate medium is selective, many bacteria other than soft rot coliforms can grow on it). When rain ceased there was a corresponding fall in the total bacterial count. It seems that as a general principle, rain is a major generator of the atmospheric bacterial aerosol. Although literature on this subject mentions rain splash as a probable aerosol generator (cf. Gregory 1973), it does not seem to have been clearly established up to now that impact of raindrops (or overhead sprinkler water drops) on to surfaces can project large numbers of bacteria into the open air. By analogy with what we have found for soft rot coliforms, it seems possible that any bacterial plant pathogen associated with above ground plant organs could be aerosolized by rain or overhead sprinklers. This has already been demonstrated for *Pseudomonas glycinea* infection of soya bean (Venette & Kennedy 1975). Whether aerosol transmission will prove to be a major influence in the spread of various diseases depends on many factors, including the meteorological conditions and the viability of the particular organism in small particles in the open air. Likewise any micro-organisms on plant surfaces could be aerosolized, and it may be that a considerable part of the airborne bacterial population in the open (particularly in rural areas) is made up of organisms derived from populations found on the surfaces of above ground plant parts.

References

ANDERSEN, A. A. 1958 New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology* **76,** 471–484.

DYE, D. W. 1969 A taxonomic study of the genus *Erwinia* II. The 'carotovora' group. *New Zealand Journal of Science* **12,** 81–97.

ERRINGTON, F. P. & POWELL, E. O. 1969 A cyclone separator for aerosol sampling in the field. *Journal of Hygiene, Cambridge* **67,** 387–399.

GRAHAM, D. C. 1972 Identification of soft rot coliform bacteria. *Proceedings of the Third International Conference on Plant Pathogenic Bacteria 1971* pp. 273–279. Wageningen: PUDOC.

GRAHAM, D. C. & HARDIE, J. L. 1971 Prospects for control of potato blackleg disease by the use of stem cuttings. *Proceedings of the Sixth British Insecticide and Fungicide Conference* **1,** 219–224.

GRAHAM, D. C. & HARRISON, M. D. 1975 Potential spread of *Erwinia* spp. as aerosols. *Phytopathology* **65,** 739–741.

GRAHAM, D. C., QUINN, C. E. & HARRISON, M. D. 1976 Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967–74. *Potato Research* **19,** 3–20.

GRAHAM, D. C., QUINN, C. E. & BRADLEY, L. F. 1977 Quantitative studies on the generation of aerosols of *Erwinia carotovora* var. *atroseptica* by simulated raindrop impaction on blackleg-infected potato stems. *Journal of Applied Bacteriology* **43,** 413–424.

GRAHAM, D. C., QUINN, C. E., SELLS, I. A. & HARRISON, M. D. 1979 Survival of strains of soft rot coliform bacteria on microthreads exposed in the laboratory and in the open air. *Journal of Applied Bacteriology* **46,** 367–376.

GREGORY, P. H. 1973 *The Microbiology of the Atmosphere* 2nd edn. London: Leonard Hill Books.

HARRISON, M. D., QUINN, C. E., SELLS, I. A. & GRAHAM, D. C. 1977 Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to re-contamination of pathogen-free potato stocks. *Potato Research* **20,** 37–52.

MAY, K. R. 1967 Physical aspects of sampling airborne microbes. In *Airborne Microbes, 17th Symposium of the Society for General Microbiology* ed. Gregory, P. H. & Monteith, J. L. pp. 60–80. Cambridge: Cambridge University Press.

MAY, K. R. & DRUETT, H. A. 1968 A microthread technique for studying the viability of microbes in a simulated airborne state. *Journal of General Microbiology* **51,** 353–366.

PEROMBELON, M. C. M. 1977 A preliminary assessment of the spread of blackleg and soft rot bacteria by haulm pulverization. *Proceedings of a Symposium on Problems of Pest and Disease Control in Northern Britain* ed. Fox, R. A. pp. 39–40. Dundee: Scottish Horticultural Research Institute.

STEWART, D. J. 1962 A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. *Nature, London* **195,** 1023.

VENETTE, J. R. & KENNEDY, B. W. 1975 Naturally produced aerosols of *Pseudomonas glycinea*. *Phytopathology* **65,** 737–738.

Soft Rot Erwinia Bacteria in Ditches, Streams and Rivers in South-East Scotland

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Using an anaerobic enrichment method, the soft rot coliform bacterium Erwinia carotovora var. carotovora was commonly found in water from field drains, ditches, streams and rivers, mainly in south-east Scotland, in mountainous, upland and arable areas. Many sites were remote from susceptible or diseased crops. The potato blackleg bacterium, E. carotovora var. atroseptica, was isolated on only one occasion.

As part of a general investigation into sources of soft rot Erwinia spp. in the environment, from which the organisms might spread and contaminate Erwinia-free potato stocks, a study was begun into the occurrence of Erwinia in association with weed roots. In late spring 1980, it was found by random testing that the roots of aquatic weeds in ditches commonly yielded these organisms, and that the water was also contaminated. By contrast, roots of weeds in adjacent fields of arable crops were not contaminated. As a result, an investigation was made into the presence of Erwinia in the water of field drains, ditches, streams and rivers over the period early June to late 1980, mainly in south-east Scotland.

Although generally considered a rainy country, many parts of the Scottish potato growing areas experience a moisture deficit in the growing season and irrigation is rapidly becoming widely practiced. Much of the irrigation water is obtained from dammed ditches, streams and rivers, which might be sources of contamination. Many streams and rivers rise in mountainous or upland areas where arable crops are not grown, and water from these sites was also examined.

In this connection, one of us (D.C.G.) made extensive tests for the presence of Erwinia spp. in waters from various sources over the years 1959 to 1962, but the method used was unsatisfactory by modern standards because no good enrichment or selective media for soft rot Erwinia were available at that time, and no Erwinia spp. were isolated (Graham and Hardie 1971). However, since these media have now been developed there was a better chance of detecting the bacteria.

Materials and Methods

Sites

Eleven ditches and drains, 20 streams and 11 rivers were tested. Their location is shown on the map (Fig. 1). They varied from field drains, small and large ditches, streams and rivers in arable lowland, to streams and rivers in upland and mountainous areas. In some cases the ditches were adjacent to potato crops. A borehole (about 40m deep) and its reservoir in an arable area and another reservoir, in a mountainous area, were also examined.

Sampling

Water was collected directly into sterilized 125 ml glass bottles. In deeper water, the sample was collected near the surface. Samples were taken several times over the period early June to late September at some sites, whereas only one sample was taken at others. Dates are given in Table 1.

Media, Isolation and Identification Methods

An equal volume of water (usually c. 50 ml) was added to double strength polygalacturonate enrichment medium (Burr & Schroth 1977) and incubated for 48h at 26° anaerobically in Gaspak jars (BBL Division, Cockeysville, Maryland, United States). A loopful of medium was plated on double layer MacConkey pectate medium (Stewart 1962) and after 48h aerobic incubation at 26° two of the characteristic Erwinia colonies were picked from each plate, transferred to nutrient agar slopes, and purified by replating if necessary. On occasions when the MacConkey pectate plates were heavily contaminated with other organisms, colonies were transferred to potato slices and rotted material diluted and plated again on MacConkey pectate medium (Graham et al. 1976). The bottles were then incubated aerobically for another 48hr, and another sample plated. On occasions, Erwinia bacteria were isolated only from the second plating, sometimes in almost pure culture.

Quite frequently no Erwinia colonies would be seen in the streak-out on the plates, but on carefully scraping away the growth on the rub-up, depressions were present in the pectate gel. After incubating for another 24h, colonies often developed in the depressions; this growth could then be purified by replating or transferring to potato slices, as mentioned above.

Results

Results are summarised in Table 1. Almost all the sites (41 out of 45) and most of the samples (81 out of 90), including those from upland or mountainous terrain, yielded E. carotovora var. carotovora. Somewhat surprisingly, E. carotovora var. atroseptica was isolated only once (from a stream) and not from water samples taken close to potato crops with visible symptoms of blackleg disease and where field drains discharged into the water course. E. carotovora

var. carotovora was obtained without difficulty throughout the sampling period, and it is noteworthy that the organism was found before potato crops had emerged on some of the high land. The pH of the water did not seem to have any influence on the presence of the organism; the pH varied from 4.6 to 8.2, the more acid water from mountainous areas having the brown colouration due to the presence of humic substances derived from peat. Water temperatures varied from 8.0°C to 18.0°C, most being in the range 12.0°C to 15.0°C. These temperatures would be suitable for survival and perhaps also multiplication of the organism.

The water from the borehole was not contaminated, but its reservoir contained E. carotovora var. carotovora, as did water from the reservoir in a mountainous area.

Discussion

The presence of Erwinia carotovora var. carotovora so commonly in waters from many different places was unexpected, especially in upland and mountainous terrain where sheep farming is the only kind of agriculture. There are scattered reports of the isolation of what were or could have been soft rot Erwinia spp. in the literature concerning water bacteriology, particularly in relation to faecal contamination (Bonde 1977), though few workers have tested their isolates for ability to produce soft rot or liquefy pectate gel. In connection with studies on the presence of coliforms in farm water supplies in the 1950s, several British workers examined water from wells, springs, ditches, streams, rivers and rain water in storage tanks for pectolytic organisms (Thomas and Thomas, 1954; Jones, 1956). Although not subjected to tests which fully characterize the bacteria, a few isolates were almost certainly Erwinia as they were non-fluorescent motile Gram-ve rods which grew in MacConkey broth at 30°C, produced acid from a range of carbohydrates, liquefied gelatin and pectate gel, and utilized citrate as a sole carbon source. They were the usually MR-ve, VP+ve. Jones and Baker (1955) isolated pectolytic bacteria from outlet water of watercress beds. One of us (D.C.G.) received three of these cultures from Mr G. Elis Jones in 1959; all were obtained during winter, in the county of Kent. When

examined by the tests now used to characterize Erwinia (Dye, 1969; Graham, 1972), two were found to be E. carotovora var carotovora and one probably E. carotovora var atroseptica (in the latter case the organism was found to be unable to produce blackleg in potato in 1971, having been in culture since 1953).

Regarding the sources of the bacteria in the present experiments, their origin is unknown, but plainly they cannot be present in the water in mountainous and upland areas as a result of seepage from infected crop plants, which could be sources in arable areas. One possibility is that rain washes out bacterial aerosols which are known to be present in the atmosphere in Scotland during summer and autumn (Quinn et al. 1980), and this is being studied. Furthermore, it may be that ditches, streams and river beds contain environments where the physical structure, chemical composition, pH and redox potential are favourable for survival of facultatively anaerobic, heterotrophic bacteria for considerable periods of time if not indefinitely. In this connection it is notable that in some cases, organisms were isolated from ditches and streams at times of year when the organisms cannot be isolated from the atmosphere, and hence could not have fallen in raindrops (Quinn et al. 1980). It is clear that the ecology of these bacteria is complex, and needs much more study. A better understanding of their occurrence in different kinds of terrain might ultimately lead to practical means of avoiding re-infection of Erwinia-free potato stocks.

Another important feature is that E. carotovora var. atroseptica was rarely isolated. This may reflect the fact that this organism is less robust than E. carotovora var. carotovora, as for instance, it does not generally survive so long in soils (Lazar and Bucur, 1964), or in aerosol particles in the atmosphere (Graham et al. 1979). Moreover, E. carotovora var carotovora is usually the soft rot coliform which contaminates Erwinia-free potato stocks during their first year in commerce (Graham et al. 1976; Perombelon et al. 1976) but from the point of view of potato blackleg control E. carotovora var atroseptica is the organism most frequently associated with this disease in the Scottish environment (Graham and Dowson, 1960; Perombelon, 1973).

The occurrence of both E. carotovora var. carotovora and E. carotovora var atroseptica in water supplies used to irrigate farm crops points to the need for farmers and growers to consider installing water purifying apparatus. This is particularly important in the case of pathogen-free potato stocks, but also where any other susceptible crops, such as brassicas, are irrigated. Equipment designed to prevent or greatly reduce contamination by faecal coliforms should also remove soft rot coliforms; these include filtration apparatus and devices to treat water with ultra-violet light (Caddy & Hurst 1978; Adams & Robinson 1979). However in tests reported by Adams and Robinson (1979), there was some evidence that soft rot Erwinia were rather less susceptible to UV light than other common water bacteria.

Table 1

Water source, date of testing in 1980 and test results for presence of soft rot Erwinia.

Site number	Water Source and terrain	Date Sampled and Result
1	Ditch A	2/6+, 16/6+, 30/6+, 21/7+, 11/8+, 1/9+
2	Ditch A	30/6+, 21/7+, 11/8+, 1/9+
3	Stream A	30/6+, 21/7+, 11/8+, 1/9+
4	Reservoir U	21/7−, 1/9+
5	Stream A	29/7+, 19/8+, 10/9+
6	Stream A	29/7+, 19/8+, 10/9+
7	Borehole A	29/7−, 10/9−
8	Stream A	29/7+, 19/8+, 10/9+
9	Stream A	29/7+, 19/8+, 10/9+
10	River A	29/7+, 19/8+, 10/9+
11	River A	29/7+, 19/8+, 10/9+
12	Stream A	29/7+, 19/8+, 10/9 +
13	River A	29/7+, 19/8+*, 10/9+
14	Reservoir A	19/8+, 10/9+
15	Drain A	19/8+, 10/9+
16	Stream M	10/9 −
17	Stream A	4/8+, 10/9+
18	Ditch A	1/7−, 22/7+, 12/8+, 2/9+
19	Stream A	9/7−, 23/7−, 7/8+, 28/7+, 18/8+, 8/9+
20	Ditch A	9/7−, 23/7+, 7/8+, 28/7+, 18/8+, 8/9+
21	Ditch A	31/7+
22	Ditch A	31/7+
23	Drain U	4/8+
24	River U	4/8+
25	Stream U	4/8+
26	River U	4/8+
27	River M	4/8−
28	Ditch M	4/8+
29	Stream M	4/8+
30	River M	4/8+
31	River M	4/8+
32	Stream M	4/8+
33	Stream M	4/8+
34	Ditch U	25/8+
35	Ditch U	25/8+
36	Stream U	25/8+
37	Stream M	25/8+
38	Stream M	25/8+
39	Stream M	25/8−
40	Stream A	25/8+
41	Stream A	27/7+
42	Stream M	27/7+
43	River U	27/7+
44	River A	27/7+
45	River A	27/7+

: A = in arable area; U = upland terrain; M = mountainous terrain. * = E. carotovora var atroseptica isolated as well as E. carotovora var. carotovora.

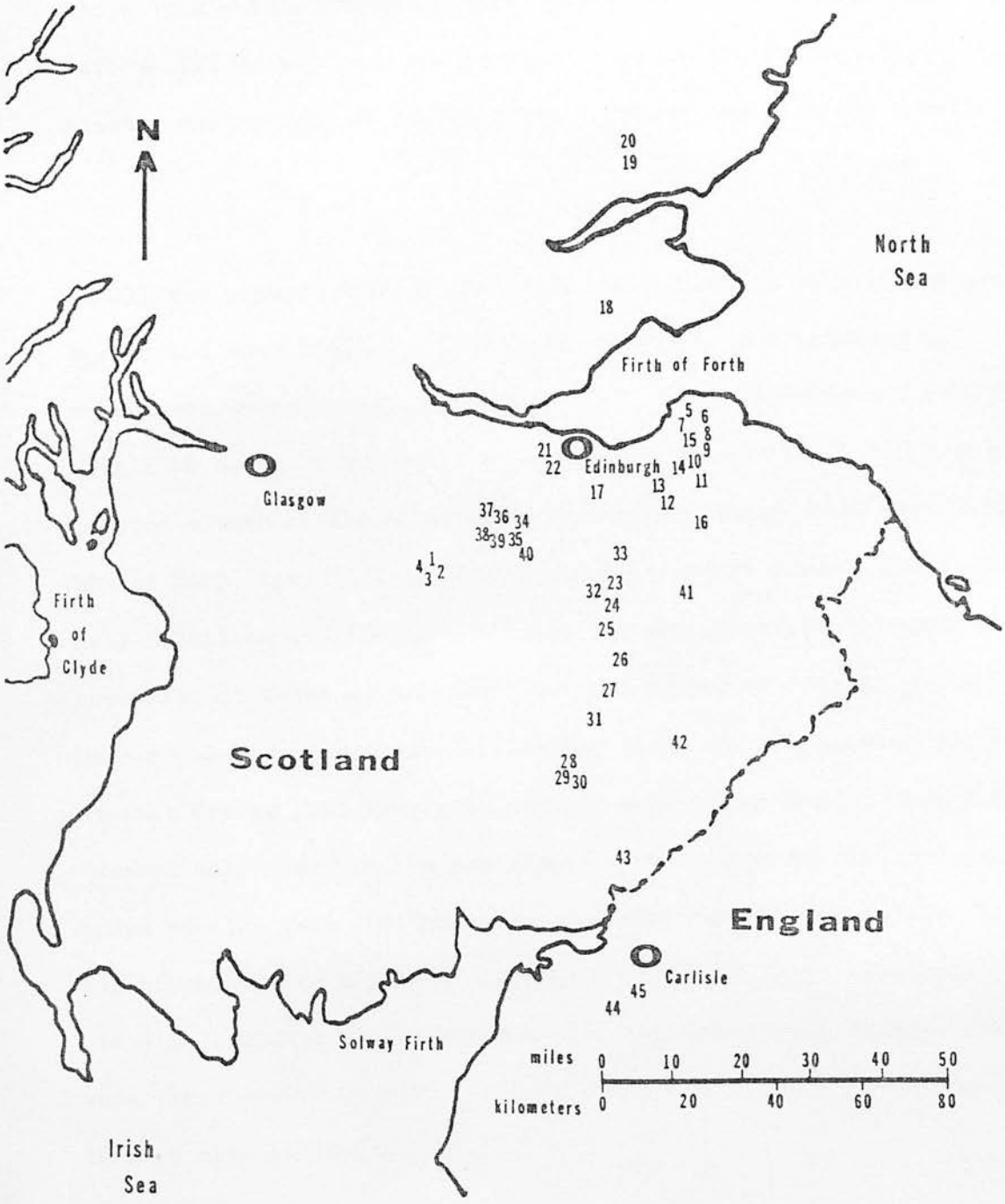


Fig. 1. Map showing water sampling sites.

THE EFFECTS OF ORGANO-MERCURY TREATMENT OF SEED POTATO TUBERS IN RESPECT OF TUBER DISEASE CONTROL AND CROPPING

Since 1960 eleven extensive experiments have been carried out to provide information on two separate issues of the treatment process, 1) tuber disease control and 2) the subsequent growth and yield of treated seed.

PROCEDURE

In all the experiments chemical treatment followed washing (by pressure spray) and consisted of a 12 minute immersion in a solution of methoxyethylmercury chloride and wetter. The experiments were started within 48 hours of harvest, except where stated, and the tubers were treated either at the premises of Scottish Treated Seeds Ltd, Eassie, or at the DAFS, Agricultural Scientific Services Station, East Craigs. The only significant difference between the installations for washing and treatment at these places concerned the method of sizing: at Eassie tubers were spool-graded between the washing phase and the dipping phase whereas at East Craigs seed had to be separated by hand-riddling before washing. "Washed only" and "washed and dipped" tubers were stored in open potato trays as also were the immediate controls, tubers hand-riddled to size but not otherwise treated. Usually a further control treatment, pitting, was also included for comparison with the traditional method: here tubers were first pitted in bulk and then stored in trays following hand-riddling to seed size in January.

Diseased tubers were hand-picked from the trays and counted and weighed on 2-3 occasions in late winter and just before planting.

To record growth and yield field experiments were conducted usually with 720 sound setts per treatment. Depending on the number of treatments, either the randomised block or latin square design was used, the usual lay-out being of 120 plants per plot, replicated six times; each plot of this kind occupied four drills of which only the middle two were employed for yield records.

The work involved in each experiment is detailed as follows:-

1. distribution of the newly-harvested tubers among the treatments and assessment of immediate losses through injury and disease;
2. assessment of losses in storage;
3. measurement of sprouting;
4. selection and weighing of sound seed for planting;
5. assessment of time and rate of emergence;
6. assessment of botanical type (achieved by measuring plant height and stemminess in mid-July - a week or two after full emergence);
7. assessment of blackleg throughout the period of growth;
8. comparison of the yields of blackleg-affected and healthy plants
9. analysis of plot yields in terms of ware, seed and chats;
10. assessment of plant yields corrected for blackleg by:-
    a. correlating the incidence of blackleg infection with plot yields and/or
    b. estimating the yield per healthy plant (ie eliminating the effects of blanks and allowing for blackleg).

(The object of 10. is to discover any yield effect not attributable to blackleg infection).

DETAILS OF EXPERIMENTS

The following experiments comprise this series to date

1. 1960-61. Arran Pilot: a comparison of the treatments, pitted, riddled, washed, and washed and dipped: treatment at Eassie, grown at Terrington (in collaboration with NAAS).

2. 1960-61. Majestic: as 1.

3. 1961. Majestic: a comparison (after pitting until mid-February) of the treatments, riddled, washed, and washed and dipped and according to whether the tubers were still dormant (late February) or sprouting (late March) at treatment: treatment and growing at East Craigs.

4. 1961-62. Arran Pilot: a comparison of the treatments, pitted, riddled, washed, and washed and dipped: treatment at East Craigs, grown at Terrington (in collaboration with NAAS).

5. 1961-62. Majestic: as 4, but grown at East Craigs and not in collaboration with NAAS.

6. 1961-62. Arran Pilot: a comparison of the treatments, pitted, riddled, washed, and washed and dipped, the mercury concentration for the dipping treatments being at 50-60 ppm (normal concentration being about 100 ppm): treatment and growing at East Craigs.

7. 1961-62. Majestic: as 6.

8. 1961-62. Majestic: a comparison of the treatments, riddled, washed, and washed and dipped (100 ppm of mercury), washed and dipped (50-60 ppm mercury), washed and dipped (100 ppm of tin-in tributyltin acetate): treatment and growing at East Craigs.

9. 1962-63. King Edward: a comparison of the treatments, pitted, riddled, washed, and washed and dipped; treatment and growing at East Craigs.

10. 1962-63. Majestic: an experiment on the effects of different water pressures for washing and comparing the treatments, pitted, riddled, hand-washed, and washed at pressures respectively of 10, 70 and 120 lbs per square inch.

11. 1962-63. Majestic: an experiment on the effect of dipping tubers in formalin before washing and comparing the treatments, pitted, riddled, washed, washed and dipped (mercury), dipped in formalin then washed and dipped in formalin then washed and dipped (mercury).

The series started in collaboration with Sir Thomas Wedderspoon and NAAS Investigation of the results of these first general experiments, together with other observations, indicated that in particular two details of the disinfection process required further attention. These were, 1. the predisposing effect of the pressure washing phase to blackleg, and 2. the differential effect of exposure to organo-mercury salts on the sprouting and growth behaviour of some varieties. Experiments 10 and 11 were designed by Dr Graham to investigate further the first of these details; experiment 3 has a bearing on the second but this question is being looked at more closely by Dr Graham in another series of experiments.

Plant Pathology Section
22 July 1963

EXPERIMENT 6 - 1961-62 - ARRAN PILOT

Treatments: pitted, riddled, washed, washed and dipped

Experimental tubers harvested: 18.10.61

Treated: 19-20.10.61

Planted at East Craigs: 10.4.62

Harvested: 22.8.62

1. Selection

Bulk was received from the field in half-hundredweight bags which were divided at random equally into four lots comprising $7\frac{1}{2}$ bags for each treatment. Except for 'pitted', the treated tubers were first dried in potato trays in a glasshouse; the temperature was maintained at $70^{\circ}F$ for about 24 hours, then reduced to $50^{\circ}F$ for 6 days. For the remainder of the storage period, trays were kept in a capacious loft where the temperature was maintained at a minimum of $35^{\circ}F$ by thermostatically controlled heaters.

The pitted tubers were included with ware from the same stock, separated by string netting. The pit was opened about nine weeks later, the seed tubers riddled from the bulk and these stored in trays along with the others.

2. Losses in storage

The losses throughout the storage period are detailed in table 1 (see separate sheet).

The tubers were not very seriously affected by rot diseases, and a considerable proportion of the losses occurred through mechanical and physical defects. Apart from these defects and excluding disease found affecting tubers at harvest, the percentage wastage in the different treatments was:- pitted - 10.3; riddled - 5.6; washed 7.2; dipped 5.5. Dry rot and gangrene

Table 1. Relative losses of seed tubers from time of harvest to planting (number of tubers)

Treatment	Time*	Mechanical plus chemical damage	Growth cracking	Insect damage	Blackleg plus jelly end rot	Blight	Dry rot	Gangrene	Soft rot	Other causes	Total waste	Total sound tubers	Percentage waste
Pitted	1						41	12		4			
	2	22	38	27	37	11	6	11	1	1	211	988	17.6
	3												
	Total	22	38	27	37	11	47	23	1	5			
Riddled	1	2		1	3	3	6	3					
	2	48	49	24	22	5	9			3	178	787	18.4
	3												
	Total	50	49	25	25	8	15	3		3			
Washed	1				3		6			1			
	2	30	41	18	19	3	25	9	1	1	162	852	16.0
	3							4	1				
	4												
	Total	30	41	18	22	3	31	13	2	2			
Dipped	1			1	1			1					
	2	45	28	14	33	2	13	1			139	793	14.9
	3												
	Total	45	28	15	34	2	13	2					

*1 - at harvest; 2 - by February; 3 - before planting

Occurred mostly in the pitted treatment but was much less in the riddled and also dipped though disinfection did not eliminate infection particularly in the case of dry rot.

3. Sprouting characteristics

Only one record of sprout growth was made - on 6th April using a random sample of 50 tubers per treatment. The records are summarised below in table 2.

Table 2. Sprouting characteristics per treatment

Treatment	Mean number of sprouts per tuber	Mean number of sprouts over 1 in. long per tuber
Pitted	5.50	1.48
Riddled	6.82	0.82
Washed	5.16	0.88
Dipped	5.64	1.30

The riddled treatment appeared to have stimulated eye development whereas pitted and dipped had the largest number of long sprouts. There was no evidence that washing or disinfection had increased sprouting in this variety as occurred in Experiment 1.

4. Selection of seed for planting

Since relatively little seed had been lost during storage it was possible size the tubers closely. The weights of tubers planted is given in table 3.

Table 3. Weight of tubers planted

Treatment	Total weight of seed planted (in lb.)	Mean weight per sett (in oz.)
Pitted	119.6	2.66
Riddled	115.0	2.56
Washed	112.8	2.51
Dipped	114.8	2.55

The figures indicate that correct selection had been achieved.

5. Emergence

Emergence was counted on three dates; the results are summarised in table 4.

Table 4. Percentage emergence on three dates

Treatment	Percentage emergence		
	23.5	4.6	13.6
Pitted	93	93	99
Riddled	81	93	99
Washed	64	96	95
Dipped	66	96	98

Good emergence had taken place by the 3rd week in May with the pitted material, but was somewhat slower with the riddled and was considerably delayed in the washed and dipped treatments. By 4th June the latter three treatments had given good emergence and had caught up with pitted. By 13th June pitted and riddled had proceeded to almost full emergence, closely followed by dipped, whereas the number of plants in the washed treatment actually fell. This was almost certainly due to the early appearance of blackleg disease, which had already caused death of some young plants.

6. Botanical type

An assessment was made of plant height and average number of stems of each of 10 plants chosen at random in each replicate of each treatment during the period 9th-11th July. Results are summarised in table 5.

Table 5. Plant height and number of stems as affected by treatments

Treatment	Average height per plant (in.)	Average number of stems per plant
Pitted	11.43	9.13
Riddled	12.15	8.57
Washed	11.38	7.18
Dipped	11.83	9.80

Analyses of the results showed that there was no significant difference between plant height in each treatment, but there was a significant increase in the number of stems in the pitted, dipped and riddled treatments over the washed and also of the dipped treatment over the riddles (Sig. diff. $\pm$ 1.24).

7. Incidence of blackleg

The incidence of blackleg disease was determined on three different dates. Results of counts are given in table 6. (Note: the figures are uncorrected for blanks).

Table 6. Incidence of Blackleg in three dates

Treatment	Percentage blackleg		
	23.7	6.8	17.8
Pitted	1.9	3.5	4.4
Riddled	0.6	2.2	3.2
Washed	7.8	15.8	23.2
Dipped	3.5	10.4	16.8

The effect of mechanical washing in increasing blackleg was very evident as the incidence of the disease was low both in the riddled and pitted treatments, while with washed almost $\frac{1}{4}$ of the crop was affected towards the end of the growing period. Dipping after washing again reduced the amount of the disease, but about 1/6 of the plants were still attacked.

8. Yield comparisons between blackleg and healthy plants

Comparisons were made in the same way as in Experiment 5. Yields are given in table 7.

Table 7. Comparison of average yield per plant of healthy and blackleg-infected plants

Treatment	Ware yield		Seed yield		Total	
	Blackleg	Healthy	Blackleg	Healthy	Blackleg	Healthy
Pitted	0.17	1.03	1.00	0.98	1.27	2.10
Riddled	0.17	0.98	1.12	1.14	1.44	2.22
Washed	0.21	0.58	1.05	1.12	1.35	1.77
Dipped	0.17	0.62	1.31	1.14	1.62	1.87

Most of the yield losses occurred in the ware fraction, the seed yields being much the same in all treatments. Differences between the ratios of the yields of blackleg and healthy plants in each of the four treatments were not significant.

9. Analysis of plot yields

The total yield, yield of ware (over $2\frac{1}{4}$ in.) and yield of seed ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.) in lb. have been compared as follows.

a. Total Yield

Treatment	Yield (lb.)	Statistical information
Riddled	632.0	In Analysis of Variance F = 5.76
Pitted	631.5	Error Variance = 106.0
Dipped	576.5	Significant difference between
Washed	504.0	figures in column 2, 76.01

Conclusion: Yield from 'riddled' and 'pitted' treatments significantly higher than that from 'washed' treatment.

b. Yield of ware-sized tubers (over $2\frac{1}{4}$ in.)

Riddled	206.5	In Analysis of Variance F = 5.55
Pitted	176.5	Error Variance = 47.0
Washed	139.5	Significant difference between
Dipped	117.0	figures in column 2, 50.61

Conclusion: Yield of 'riddled' treatment is significantly higher than that from 'washed' and 'dipped' treatments. Yield from 'pitted' treatment significantly higher than that from 'dipped' treatment.

c. Yield of seed-sized tubers ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.)

Treatment	Yield (lb.)	Statistical information
Riddled	423.5	In Analysis of Variance F = 9.82
Dipped	416.0	Error Variance = 33.3
Riddled	388.0	Significant difference between
Washed	326.0	figures in column 2, 42.60

Conclusion: Yield from 'washed' treatment significantly lower than that from any other treatment.

10. Estimation of yield per healthy plant (ie discounting blanks and blackleg)

A moderate negative correlation (r = -0.55) occurred between the mean total plant yield per plot and the number of blackleg plants per plot. A check was again made to see if the difference between treatments was due to the presence of blackleg disease. The yields were therefore subjected to further analyses, summarised below, in which the statistics used were the yields per healthy plant.

a. Total Yield

Treatment	Estimated yield per healthy plant (lb.)	Statistical information
Pitted	1.82	In Analysis of Variance F = 2.5
Riddled	1.79	Error Variance = 0.032
Dipped	1.68	
Washed	1.56	

Conclusion: The observed differences between the figures in column 2 are not significant.

b. Yield of ware-sized tubers (over $2\frac{1}{4}$ in.)

Riddled	0.60	In Analysis of Variance F = 2.7
Pitted	0.51	Error Variance = 0.017
Washed	0.48	
Dipped	0.38	

Conclusion: The observed difference between the figures in column 2 are not significant.

c. Yield of seed-sized tubers ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.)

Treatment	Estimated yield per healthy plant (1lb.)	Statistical information
Pitted	1.18	In Analysis of Variance F = 5.5
Dipped	1.15	Error Variance = 0.009
Riddled	1.09	Significant difference between
Washed	0.96	figures in column 2, 0.12 (ie 0.70/6)

Conclusion: Yield from 'washed' treatment significantly lower than that from any other treatment.

The results given in sections 9 and 10 again show that the reduction in total yield given by the washed and dipped treatments as against pitted and riddled is likely to have been caused by the varying amounts of blackleg disease in the treatments.

The highest seed yield in this experiment was given by the pitted treatment, closely followed by dipped. This probably resulted from the significantly greater number of stems on the growing plants than those from the riddled or washed material. However, it might have been expected that since plants grown from dipped tubers had on the average rather more stems than those from pitted, the yield of seed from dipped would also have been somewhat greater whereas it was actually $7\frac{1}{2}$ lb. less. But when the number of tubers yielded by the two treatments was compared, dipped was found to have given 187 more tubers than pitted (dipped = 2826; pitted = 2639). This indicates that the average weight and therefore size of the tubers from the pitted treatment was greater than from the dipped treatment. If the produce had been graded so that the seed fraction consisted of tubers sized 2 in. - $1\frac{1}{4}$ in. (which is the more usual practice with early varieties like Arran Pilot) there seems to be a distinct possibility that the dipped treatment would have been left with a greater weight of seed than pitted as more tubers from pitted would have passed into the ware category because of their greater size.

Plant Pathology Section
25th March 1964

EXPERIMENT 7 - 1961-62 - MAJESTIC

Treatments: pitted, riddled, washed, washed and dipped

Experimental tubers harvested: 18.10.61

Treated: 20.10.61

Planted at East Craigs: 10.4.62

Harvested: 18.10.62

1. Selection

Bulk was received from the field in 28 half-hundredweight bags, which were divided at random into four lots of 7 bags per treatment. The four portions were dealt with and stored in the same way as the Arran Pilot in Experiment 6.

2. Losses in storage

Storage losses are summarised in table 1 (see separate sheet).

The tubers were attacked by both dry rot and gangrene, the diseases being fairly well controlled by disinfection, although washing alone caused a considerable increase in gangrene. Washing also brought about considerable losses from bacterial soft rot. Losses in the washed and dipped treatment from mechanical and chemical damage were considerable - chemical damage was extensive in the dipped treatment because tubers were somewhat susceptible to skinning during washing in the machine.

Apart from mechanical and physical defects and excluding disease at harvest, the percentage wastage in the different treatments was:- pitted - 14.1, riddled - 9.0, washed 22.8, dipped 4.2.

3. Sprouting characteristics

On 6th April an assessment of living eyes was made using 50 tubers per treatment. The eyes had not produced obvious sprouts at this time. Results are given in table 2.

Table 1. Relative losses of seed tubers from time of harvest to planting (number of tubers)

Treatment	Time*	Mechanical plus chemical damage	Growth cracking	Insect damage	Blackleg	Blight	Dry rot	Gangrene	Soft rot	Scab	Other causes	Total Waste	No. of sound tubers	Percentage waste
Pitted	1													
	2	22	42	3	1	6	92	26						
	3	3					35	22		1		253	1043	19.5
	Total	25	42	3	1	6	127	48		1				
Riddled	1	1			8	1				1				
	2	16	24	5	1	2	52	19	1	3				
	3						26	11				171	1113	13.3
	Total	17	24	5	9	3	78	30	1	4				
Washed	1			2	11	5	1			1	7			
	2	43	22	4	2	2	79	78	75	4	13			
	3	11	1				14	29	6			410	917	30.9
	Total	54	23	6	13	7	94	107	81	5	20			
Dipped	1	3	1	1	13	15				4				
	2	116	25	3	1	1	15	10	2	5	9			
	3	5	9				1	5				244	926	20.9
	Total	124	35	4	14	16	16	15	2	9	9			

*1 - at harvest; 2 - by February; 3 - before planting

Table 2. Sprouting characteristics, April 1962

Treatment	Mean number of growing eyes per tuber
Pitted	4.80
Riddled	6.18
Washed	5.74
Dipped	6.40

As in previous experiments, the greatest number of developing eyes occurred in the dipped treatment, though it was closely followed by riddled. Pitted showed fewest growing eyes, and statistical analysis of the results demonstrated that pitted gave significantly less eyes than any other treatment.

4. Selection of seed for planting

A selection of tubers for size was made just prior to planting. The weights of tubers planted in each treatment is given in Table 3.

Table 3. Weight of tubers planted

Treatment	Total weight of seed planted (in lb.)	Mean weight per sett (in oz.)
Pitted	122.9	2.73
Riddled	119.6	2.66
Washed	108.4	2.41
Dipped	107.9	2.40

Statistical analysis of the weights showed no significant differences between treatments.

5. Emergence

Emergence was determined on four dates; results are summarised in table 4.

Table 4. Percentage emergence on four dates

Treatment	Percentage emergence			
	7.6	15.6	25.6	2.7
Pitted	89	96	96	97
Riddled	92	98	98	98
Washed	66	80	81	83
Dipped	72	90	92	94

Good emergence had taken place by the end of the first week of June with both pitted and riddled treatments, whereas only about $\frac{3}{4}$ of the dipped tubers and $\frac{2}{3}$ of washed tubers had emerged. One week later almost all tubers which were viable had emerged in all treatments and improvements later were only fractional. Seventeen per cent of the washed tubers did not produce plants and blanking was very obvious.

6. Botanical type

An assessment was made of plant height and average number of stems on each of ten plants chosen ~~as~~ at random in each replicate of each treatment. Results are summarised in table 5; counts were made on 6th July.

Table 5. Plant height and number of stems as affected by treatments

Treatment	Average height per plant (in.)	Average number of stems per plant
Pitted	16.15	2.53
Riddled	15.93	2.67
Washed	12.92	2.27
Dipped	13.87	3.67

Analysis of the results showed that plant height in the pitted and riddled teatments was significantly greater than in washed or dipped (Sig. diff. $\pm$ 1.42). Once again there was a significantly greater number of stems in the dipped treatment than in any other treatment (Sig. diff. $\pm$ 0.41).

7. Incidence of blackleg

Incidence of blackleg was determined on five dates from 5th July to 20th September inclusive. The counts are summarised in table 6. (Figures uncorrected for blanks).

Table 6. Incidence of blackleg on different dates

Treatment	Percentage blackleg				
	5.7	20.7	6.8	29.8	20.9
Pitted	0.4	1.2	1.3	1.8	2.8
Riddled	1.5	2.1	2.9	3.3	5.3
Washed	8.0	10.1	12.8	16.9	22.4
Dipped	0.1	0.3	0.6	1.3	3.0

The development of blackleg was very marked in the washed treatment, but spread during washing was completely controlled by dipping in this case - riddled giving rather more infected plants than dipped.

8. Field comparisons between blackleg and healthy plants

Comparisons were made in the same as in previous experiments. Results are given in Table 7.

Table 7. Comparison of average yield per plant of healthy and blackleg-infected plants

Treatment	Ware Yield		Seed Yield		Total	
	Blackleg	Healthy	Blackleg	Healthy	Blackleg	Healthy
Pitted	1.25	2.67	0.37	0.95	1.62	3.66
Riddled	0.92	2.54	0.83	1.11	1.79	3.68
Washed	0.67	2.99	0.50	0.88	1.20	3.94
Dipped	0.38	2.30	1.37	1.33	1.81	3.66

Losses occurred both in ware and seed fractions in pitted, riddled and washed treatments, although in dipped the blackleg affected plants produced more seed than healthy. This was offset by the very small amount of ware produced by blackleg-affected plants. Differences between the ratios of the yields of

blackleg and healthy plants were not examined for significance as an insufficient number of diseased plants could be found in the field in each block in the pitted, riddled, dipped treatments to make the calculation meaningful.

9. Analysis of plot yields

The total yield, yield of ware (over $2\frac{1}{4}$ in.) and yield of seed ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.) in lb. have been compared as follows:-

a. Total Yield

Treatment	Yield (lb.)	Statistical Information
Pitted	881.5	In analysis of Variance F = 29.25
Riddled	869.0	Error Variance = 67.4
Dipped	808.5	Significant difference between
Washed	645.0	figures in column 2, 60.61

Conclusion: Yield from pitted treatment significantly higher than from dipped and yield from riddled almost so. Yield from dipped significantly higher than from washed.

b. Yield of ware-sized tubers (over $2\frac{1}{4}$ in.)

Pitted	688	In Analysis of Variance F = 21.93
Riddled	674	Error Variance = 65.53
Washed	522	Significant difference between
Dipped	519	figures in column 2, 59.75

Conclusion: Yield from pitted and riddled treatments significantly higher than from washed or dipped.

c. Yield of seed-sized tubers ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.)

Dipped	280	In Analysis of Variance, F = 24.16
Pitted	189	Error Variance = 27.13
Riddled	188	Significant difference between
Washed	128	figures in column 2, 38.44

Conclusion: Yield from dipped treatment significantly higher than from any other treatment. Yields from pitted and riddled treatments significantly higher than from washed.

10. Estimation of yield per healthy plant (ie discounting blanks and blackleg)

In this experiment the negative correlation (r = -0.80) between mean total plant yield per plot and the number of blackleg plants per plot was greater than in any other previous experiments. Calculations were again made to see if the differences between treatments were due to the presence of blackleg disease. Results of the analyses are as follows:-

a. Total Yield

Treatment	Estimated yield per healthy plant (lb.)	Statistical Information
Pitted	2.56	In Analysis of Variance, F = 1.0
Riddled	2.55	Error Variance = 0.03
Washed	2.49	
Dipped	2.41	

Conclusion: The observed differences between the figures in column 2 are not significant.

b. Yield of ware-sized tubers (over $2\frac{1}{4}$ in.)

Washed	2.07	In Analysis of Variance, F = 11.00
Pitted	2.01	Error Variance = 0.03
Riddled	2.00	Significant difference between
Dipped	1.56	figures in column 2, 0.21 (ie 1.28/6)

Conclusion: Yield from pitted and riddled treatments significantly higher than from washed and dipped.

c. Yield of seed-sized tubers ($2\frac{1}{4}$ - $1\frac{1}{4}$ in.)

Dipped	0.82	In Analysis of Variance, F = 16.0
Riddled	0.54	Error Variance = 0.01
Pitted	0.54	Significant difference between
Washed	0.44	figures in column 2, 0.13 (ie 0.75/6)

Conclusion: Yield from dipped treatment significantly greater than from any other treatment.

As explained earlier, blackleg occurred very markedly in the washed treatment, but only to a relatively small extent in the other three treatments. The

presence of this disease was correlated with a considerable yield reduction in washed, which gave only 73% of the yield produced by pitted seed.

The experiment is also interesting in that the percentage blackleg in dipped was about the same as in pitted and 2.3% less than in riddled. Furthermore the percentage of emerged plants in these three treatments was not greatly different, yet the total yield from dipped was significantly less than from pitted (92%) and almost significantly less than from riddled (93%). This appears to indicate that the reduction was not caused by blackleg and blanking and although mathematical corrections allowing for these two conditions gave yields which were not significantly different, dipped still produced less than pitted or riddled. It seems possible that this slight reduction is not fortuitous, but results from the mercurial treatment which causes the production of a greater number of stems and therefore more seed-sized tubers at the expense of maximum bulk. Previously this activity has been obscured by the much increased blackleg content of dipped over pitted and riddled treatments, or, in the case of Experiment 5, by the large amount of blanking in dipped.

Plant Pathology Section
11 May 1964

DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

SUMMARY DATA SHEET ON

SEC-BUTYLAMINE

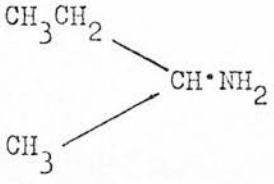
Prepared by D C Graham, 1971

1. IDENTITY

1.1 Chemical name

sec-butylamine, 2-aminobutane

1.2 Structural formula



Empirical formula

$C_4H_{11}N$

Molecular weight

73.16

1.3 Other names

None

1.4 Proprietary products containing it

Tutane™ base and Tutane™ Carbonated Solution (Elanco Products Co., division of Eli Lilly and Co.) These products are not sold in the UK.

2. PHYSICAL AND CHEMICAL PROPERTIES

2.1 Physical properties

Colourless liquid with ammoniacal odour

BP 63°C

VP 135 mm Hg at 20°C

n^{20}_D 1.394; n^{20}_4 0.724

Solubility: miscible with water and most organic solvents

Inflammable: flash point -6.5°C; lower explosive limit of sec-butylamine in air 21,000-25,000 ppm.

2.2 Chemical properties

An organic base forming water soluble salts with acids. Having an asymmetric carbon, it exists as optical isomers; the commercially available products are racemic mixtures.

Stable, but corrosive to tin, aluminium, copper and its alloys and some steels.

3. METHODS OF ANALYSIS

3.1 Product analysis

Conventional steam distillation of the amine into standard acid and back titration.

3.2 Residue analysis

Sec-butylamine is stable in association with plant tissue. It presumably occurs as the salts of cell organic acids, although a significant proportion reacts with some component (or components) of plant tissue to form a product which is not extractable with water.

The method of choice for residue determination is a modification of that described by Day et al (1). The Day method consists of the separation of volatile amines from the tissue by Kjeldahl distillation, removal of co-distilled interfering substances by a carbon tetrachloride wash, and dinitrophenylation with 2,4-dinitrofluorobenzene, followed by GLC.

In this laboratory it has been found that some changes in the method are necessary when it is applied to analysis of residues in potatoes. The CCl_4 washing step was responsible for poor resolution in the final chromatographic analysis, and was omitted. This did not result in any interference from volatile components in the potato distillate. A variety of stationary phases was used for GLC and it was found that best resolution was obtained using 6' x ¼" o.d. column packed with 3% Silicone SE 30 on Gas-Chrom Q, although Silicones QF1 and XE 60, and DEGS also gave satisfactory resolution.

3.3 Analysis in Air

Since treatment of plant material is done in closed chambers it is necessary to use a method which will give an immediate indication of the presence of sec-butylamine in air in the event of leaks. This can be carried out with Drager ammonia tubes, which will rapidly detect levels somewhat below 5 ppm up to very high concentrations.

4. APPLICATION

4.1 Types of pests controlled

Sec-butylamine is effective in the laboratory and in field experiments for

the control of fungal pathogens causing post-harvest decay of citrus, apple and peach fruits, including Penicillium spp, Botrytis spp and Thielaviopsis basicol (2).

Studies in this laboratory have shown it to be very effective in controllir the potato tuber 'latent' diseases gangrene and skin spot (caused by Phoma exigua var. foveata and Oospora pustulans respectively) when applied as a fumigant (3).

4.2 Crops to be treated

The only crop to be treated is seed potatoes during the storage period.

4.3 Formulation

Applied as the free base in gaseous form.

4.4 Application

The gaseous free base is applied to tubers held in trays, bags, boxes or bulk, in suitably designed fumigation chambers, fitted with forced draught gas recirculation systems incorporating vapourisers. Drawings of an experimental fumigation chamber and a "commercial" fumigation chamber are included (Appendix 1 Experiments using a Dutch type potato storage bin fitted for internal recirculati have shown it can easily be modified for use as a fumigation chamber. All commercial chambers would be fitted with a separate fan extraction system to remove residual sec-butylamine from the chambers before opening, as shown in Appendix 1, drawing 2.

The dosage of sec-butylamine needed to achieve good control is 200 mg/kg tubers. Tubers are best treated as soon as possible after lifting, though satisfactory control can still be obtained 2 weeks after lifting and it might be possible to extend this to 3 or 4 weeks. It may also be possible to prevent some development of gangrene on healthy tubers by fumigation after grading during January, February or March - experiments are in progress. For commercial use at this stage of development treatment will only be done within 2 weeks of lifting.

The process of application and distribution of the chemical through the tubers takes approximately 3 hrs (3). The liquid free base is introduced into the vapouriser at such a rate that the concentration of gas leaving the vapouriser is below the lower explosive limit.

4.5 Mode of action

The mode of fungitoxic action is not well understood though it is both fungistatic and fungicidal. It is not systemic. Of many aliphatic amines tested, sec-butylamine has proved the most active. Replacement of the C_1 or C_4 methyl group with CF_3, CCl_3, COOH, OCH_3, CH_2OH, Cl, NH_2 or OH resulted in inactive substances. The (-) enantiomorph has been found to be considerably more active than the (+), both in preventing spore germination and in inhibiting mycelial growth.

The receptor site on the fungus cell appears to consist of an anionic component which binds to the $-NH_3^+$ group, and a hydrophobic area which is complementary to the sec-butyl radical as spatially oriented in (-) sec-butylamine (4).

4.6 Phytotoxicity

No phytotoxicity occurred at the recommended dosage rate apart from slight browning of tissue below skinned areas of immature potato tubers. The phytotoxic dose (causing extensive skin necrosis and death of eyes) lies between 1000 and 5000 mg/kg (3).

RESIDUES

5.1 Residue data

Residues in fresh material

Residue studies have been carried out on main crop potatoes of several cultivars treated after harvesting in October/November 1967, '68, '69 and '70. Residue analyses were also made on immature early potatoes harvested in July/August 1969. In 1967 the treatments were carried out at a dosage of 140 mg/kg but all subsequent treatments were carried out at 200 mg/kg. After treatment,

crops were stored either in bulk, surrounded by straw in an unheated building, or in open wooden boxes in a building maintained at 5-6°C. At varying intervals over a period of several months following treatment, samples were taken from different positions in the store, selecting only whole tubers with no obvious signs of mechanical damage. For analysis, four tubers from each sample were quartered and quarters from each tuber were macerated together to a homogenous pulp. A 50 g sample of this pulp was used for the analysis (which was completed by the method given in section 3.2). The results of residue analyses of whole tubers are summarised below.

Year	Time of treatment	No. of cvs.	No. of stocks	No. of analyses	Range of residues (ppm)	Mean residue (ppm)
1967	Within 5 days of harvest	2	2	4	11-22	16
1968	Within 3 days of harvest	5	9	40	33-194	81
	14 days after harvest	2	3	8	33-71	43
1969	Within 3 days of harvest	5	5	66	22-241	83
	14 days after harvest	2	2	18	21-229	66
	Immature early varieties, within 3 days of harvest	2	3	64	47-389	164
1970	Within 3 days of harvest	2	2	26	10-236	103

The lower residues resulting from the 1967 treatments can be attributed to the lower dosage and inefficient means of application in the early trials. In 1969 an attempt was made to study any decline in residue levels over a period of 5 months from the time of treatment. Samples for analysis were taken immediately following treatment and at 4 week intervals during subsequent storage in an unheated building. The wide range of the residues found at any one time made it impossible to follow any pattern of general decline in residue levels, though there was no evidence for any substantial decline during storage.

Residue analyses were carried out on the uncooked flesh and peel of treated tubers following peeling using a domestic hand potato peeler. The distribution of the residues between peel and flesh is given below. In all cases the flesh represented approximately 90% of the weight of a tuber.

Year	Time of treatment	No. of cvs.	No. of stocks	No. of analyses	Range of % residue in flesh	Mean % residue in flesh
1968	Within 3 days of harvest	3	3	6	22-39	28
	14 days after harvest	1	1	4	14-21	19
1969	Within 2 days of harvest	3	3	9	7-51	36

The analyses gave a range of residues in the peel of treated tubers from 200-1000 ppm.

Cooking Tests

Analyses were made to determine any reduction in residue levels in treated tubers after cooking by boiling in water in the usual way and also by crisping in a vegetable oil at 180°C. Tests have been carried out on both whole and peeled tubers. The tubers were halved before cooking and one half analysed uncooked and the other half analysed immediately after cooking. The results are given below

Year	No. of cvs.	No. of stocks	Range of % residue removed by cooking	Mean % residue removed by cooking
a) Cooking by boiling				
1968 (whole tubers)	3	5	24-59	38
1968 (peeled tubers)	2	2	19-51	35
1969 (peeled tubers)	2	2	39-50	44
b) Cooking by crisping				
1969 (whole tubers)	1	1	33-61	47
1969 (peeled tubers)	3	3	39-58	45

The residues in both peeled uncooked and peeled and cooked tubers although very variable are substantial, and because of this it is considered that they might be a hazard if regularly eaten by human beings or livestock. In the

circumstances it is considered that sec-butylamine should not be used on ware (table) potatoes until more data are available on toxicity.

Residues in crops grown from treated seed

In 1968, crops were harvested which had been grown from seed potatoes treated at a rate of 140 mg/kg within 3 days of lifting in 1967. In 1969 crops were harvested from seed treated at levels of 200, 500 and 1000 mg/kg within 3 days of lifting in 1968. In 1970, crops were harvested from seed treated 3 days and 14 days after lifting in 1969 at 200 mg/kg. The results of analyses of all of these crops and untreated material from the same source are given below.

Year	Treatment of seed tubers	No. of cvs.	No. of stocks	Range of residue (ppm)	Mean residue (ppm)
1968	140 mg/kg	2	2	0.05	0.05
	Nil	2	2	0.05	0.05
1969	200 mg/kg	3	4	0.04-.012	0.08
	500 mg/kg	1	1	0.09-0.16	0.14
	1000 mg/kg	1	1	0.05-0.11	0.08
	Nil	3	4	0.03-0.11	0.07
1970	200 mg/kg (3 days)	2	2	0.01-0.10	0.05
	200 mg/kg (14 days)	2	2	0.01-0.06	0.03
	Nil	2	2	0.01-0.04	0.03

The residues found in crops grown from treated seed are very small and not significantly different from those found in untreated material, even when the mother tubers had been treated at 5x the recommended dosage. There is therefore no translocation of sec-butylamine to daughter tubers, and there is no hazard to consumers of crops grown from treated seed.

All the residue figures given above were obtained using the method of analysis given in 3.2 The mean blank value obtained from analysis of untreated tubers was 0.07 ppm with a standard deviation of 0.025 ppm based on 16 analyses. The mean recovery at levels between 10 ppm and 100 ppm was 85%. The lower limit of determination of the method of analysis as it was applied in 1967 and 1968

was 0.05 ppm. Improvements in the method and apparatus in 1969 gave a lower limit of determination of 0.01 ppm. All the results presented above have been corrected by a recovery figure of 85% but have not been corrected by the blank value given above.

5.3 Persistence in soil, water etc

No tests have been made on rate of disappearance in soil etc, but it is unlikely to be persistent as experiments show sec-butylamine can be utilized as a carbon source by various soil bacteria, actinomycetes and fungi.

6. EXPERIMENTAL DATA ON TOXICITY

6.1 Toxicity to invertebrates

No information, though not relevant to proposed use.

6.2 Acute toxicity to vertebrates

6.2.1 Other than mammals

	Sec-butylamine free base pH 13.0 100%	Sec-butylamine carbonated pH 8.5 15.45%
Bluegill fish LC_{50}	50 ppm 100 ppm	100 ppm
Hen, oral LD_{50}	250 mg/kg	1734 mg/kg

6.2.2 Mammals

Rat, oral LD_{50}	380 mg/kg	8000 mg/kg
Dog, oral LD_{50}	250 mg/kg	3468 mg/kg
Rabbit, dermal LD_{50}	2500 mg/kg	2500 mg/kg
Rabbit, irritation	strongly irritant	not irritant
Rat, inhalation LC_{50}	3.5 mgL/hr	-

(Source, Ref. 2)

Experience has shown that hazards relating to its application are due primarily to its alkalinity. Like ammonia, these effects are minimized both with dilution and as the basicity is neutralised (2). According to Sax (5), sec-butylamine presents a moderate acute local toxic hazard as an irritant and on ingestion and inhalation. The acute systemic effects are not described. Sax (5) gives the same toxic hazard rating to n-butylamine, which has been somewhat better investigated (oral LD_{50} rat 500 mg/kg). It is a severe skin and eye irritant. The systemic effects in rats were restlessness, excitability,

increased pulse and respiratory rate, dyspnoea, convulsions and death (5). The recommended threshold limit for n-butylamine in industry, based on analogy to ethylamine and unpublished industrial experience is 15 mg/m^3 air (7). With sec-butylamine treatments concentrations of this level have never been reached in the air of the general working space surrounding potato fumigation chambers, though concentrations above this level have been detected with Drager NH_3 tubes very close to points where gas has leaked from the chambers.

6.3 Cumulative and chronic toxicity

Feeding studies have shown that sec-butylamine and its salts have a low order of cumulative and chronic mammalian toxicity.

In 3 month feeding tests using the acetate salt, no injury of any kind occurred to rats fed at 10,000 ppm or dogs fed at 5,000 ppm of their daily food intakes (8).

In long term (2 year) feeding and reproduction studies on rats, 2500 ppm of free base or acetate salt in the diet was found to be a safe level. Similar feeding studies on dogs showed 5000 ppm to be safe (2) though Martin (9) gives only 2500 ppm. No data on pathological changes are available.

6.4 Metabolism

No data are available, except that cattle fed with citrus pulp containing sec-butylamine salts produce milk containing extremely low residues of unchanged chemical (10).

6.5 Toxicity to man

There are no reports of occupational injury from inhalation of sec-butylamine vapour. Two persons experimenting on potato fumigation with sec-butylamine on a large scale have been exposed intermittently to vapour over a 10 week period each year for 4 years without any ill effects. Several other persons have handled substantial amounts of potatoes treated at a dose of 200 mg/kg over a 4 year period without signs of skin irritation or other effects. A female worker handling tubers immediately after treatment at a high dosage (c. 1000 mg/kg) complained of slight eye irritation.

7. MEDICAL DATA

7.1 Diagnosis of poisoning

The symptoms of poisoning in man are unknown.

7.2 Treatment of poisoning

The danger from sec-butylamine arises when handling the concentrated free base, since high concentrations of vapour irritate the respiratory system and eyes and the liquid is assumed to be very irritant and poisonous if taken by mouth in quantity (11).

The following treatments are given in Gray (11).

Vapour inhaled: remove from exposure, rest and keep warm; in severe cases, obtain medical attention.

Affected eyes: irrigate thoroughly with water; in severe cases or where splashing has occurred, obtain medical attention.

Skin contact: drench with water; remove and wash contaminated clothing before re-use.

If swallowed: wash out mouth thoroughly with water and give plenty of water to drink; obtain medical attention.

8. FIELD OBSERVATIONS

None, but animals or birds eating treated potatoes planted in the field should not be harmed.

9. PERMITTED USE IN OTHER COUNTRIES

Available in the United States from Elanco Products Co, but apparently its commercial use has not developed, probably because ammonia can be used successfully to control storage rots of citrus, and also because residues can be detected in milk of cattle fed with processed citrus pulp prepared from treated citrus fruits.

As a result no permanent residue tolerances have been adopted by the United States FDA, but an experimental tolerance of 20 ppm has been granted for commercial scale experiments on apples and citrus fruits (12).

REFERENCES

1. DAY, E W et al., J. Assoc. off. anal. Chem., 51, 39, 1968
2. ANON., Technical Report on Tutane, Blanco Products Co, 1966.
3. GRAHAM, D C and HAMILTON, G A, Nature 227, 297, 1970.
4. LOCKERT, J W et al., Phytopathology (abs.) 60, 1533, 1970.
5. SAX, N I, Dangerous Properties of Industrial Materials, 3rd edition. Reinhold Book Corporation, 1968.
6. MARZLIK, P J, J. Pharm. exp. Ther., 20, 435, 1923.
7. PATTY, F A (ed), Industrial Hygiene and Toxicology, Vol 2, Interscience, 1963.
8. LOCKERT, J W and KOLBEZEN, M J, Phytopathology, 54, 978, 1964.
9. MARTIN, H, 2-Aminobutane, World Review of Pest Control (Suppl.), Summer 1969.
10. SHERRY, J A, Personal communication, 1968.
11. GUY, C M (ed), Laboratory Handbook of Toxic Agents, The Royal Institute of Chemistry, 1960.
12. ANON, Extension of temporary experimental tolerance for 2-aminobutane in apples, lemons and oranges, Fed. Reg. 31, 14851.

APPENDIX 5

MINISTRY OF AGRICULTURE FISHERIES AND FOOD
DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

SAFE AND EFFICIENT FUMIGATION PRACTICE

THE SAFE USE OF 2-AMINOBUTANE

FOR FUMIGATION OF POTATOES

September 1977

THE SAFE USE OF 2-AMINOBUTANE FOR THE FUMIGATION OF POTATO TUBERS

INTRODUCTION

1. 2-Aminobutane (or sec-butylamine) is a colourless or pale straw-coloured liquid with an ammoniacal odour. It is used in the chemical industry for various purposes, but its antifungal activity was first discovered in the United States in the early 1960's. Treatment of potato tubers with 2-aminobutane for fungal disease control was developed by the Agricultural Scientific Services of the Department of Agriculture and Fisheries for Scotland. The chemical is particularly effective against skin spot (Oospora pustulans) and gangrene (Phoma exigua var. foveata) and shows some activity against silver scurf (Helminthosporium atrovirens); it has no effect on dry rot (Fusarium caeruleum), blight (Phytophthora infestans) or black scurf (Rhizoctonia solani). Its action on fungi causing other potato tuber diseases is not known.

2. Because of the potentially hazardous nature of 2-aminobutane it must only be used as a fumigent of potatoes in fumigation chambers designed for the purpose, or in potato stores which have been specially modified to allow the fumigation process to be safely conducted. In all cases, and before use, the fumigation plant (and buildings or chambers where appropriate) must be inspected and tested, and the procedures to be followed approved by either the Department of Agriculture and Fisheries for Scotland (in Scotland) or the Ministry of Agriculture, Fisheries and Food (in England and Wales).*

RESPONSIBILITIES OF EMPLOYERS AND EMPLOYED PERSONS

3. Employers should be aware of their responsibilities under the Health and Safety at Work, etc, Act 1974, especially those under Section 2 of that Act. They must ensure that in all operations involving the handling and use of 2-aminobutane the guidance set out in this leaflet is observed and that staff are adequately trained and are themselves familiar with this leaflet. In particular, employers must maintain fumigation plant (and, where appropriate, the structure of associated buildings), provide the equipment necessary for safe operation, and ensure adequate management control.

4. Employees should be aware of their responsibilities under the Health and Safety at Work, etc, Act 1974, and especially those under Section 7. They must follow the advice and guidance given in this leaflet as it relates to their particular jobs and responsibilities. The operator in charge of a fumigation should ensure that correct procedures are followed and that unprotected workers are not exposed to the chemical.

5. Self-employed persons should be aware of their duties under the Health and Safety at Work, etc, Act 1974, and should follow the guidance given in this leaflet as it applies to both employers and employees.

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* Potential users of 2-aminobutane for the purpose of fumigating potatoes should contact either Chemical Spraying Company, Glenearn Road, Perth, or Wedderspoon Processes Limited, Shielhill House, Forfar, Angus, who hold the licences to operate the process in England and Wales and Scotland from the National Research Development Corporation.

6. Fumigations with 2-aminobutane must only be carried out by staff who have received instruction on the procedures to be followed and in the safety precautions necessary for handling and applying the fumigant and who are otherwise competent for the purpose. They should know how to use the respiratory protective equipment and the gas detection equipment (as may be appropriate) and be aware of first aid action to take in the event of an accident.

THE FUMIGANT

7. 2-aminobutane boils at 63°C and is soluble in water and most organic solvents. The liquid is highly flammable (flash point - 19.5°C) and the lower explosive limit in air is 2.1-2.5%. It is a mono-alkyl derivative of ammonia forming water soluble salts with acids, and has a strong unpleasant ammoniacal smell, but in low concentrations in air the odour is not objectionable. The chemical is stable but is strongly alkaline and corrosive to tin, aluminium, copper and its alloys, and some steels.

8. The liquid can cause skin and eye burns and the vapour is irritating to the throat and the eyes. It is assumed to be very irritant and poisonous if taken by mouth, but the symptoms of poisoning in man are unknown and there are no records of any chronic toxic action. The effects of 2-aminobutane are minimised by dilution (especially with water) and neutralization. No recommended Threshold Limit Value* has been set for 2-aminobutane but provisionally a figure of 25 ppm should be observed as a ceiling limit value (maximum allowable concentration at any time).

9. The time when fumigation achieves best control is 2-4 weeks after lifting but later fumigation is possible although effectiveness decreases as the time between harvest and treatment lengthens.

Occasionally, because of immaturity, and other reasons, the tubers can be extensively damaged during lifting and even during dressing after periods of storage. If damage does occur at any time, it is advisable to leave the potatoes to heal before treatment; the length of time required is dependent on temperature, humidity and the condition of the tubers. If potatoes are fumigated when there are areas of broken skin or mechanical damage, the fumigant will penetrate into these areas and cause chemical damage. This effect, due to the killing of the surface layers of cells, causes a blackening of the surface, and can give the tubers a poor appearance, but otherwise causes no harm.

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* Threshold Limit Values refer to airborne concentration of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed during a 7 to 8-hour workday and a 40-hour working week without adverse effect.

For a full discussion of this concept see Technical Data Note 2'Threshold Limit Values', available free of charge from any Factory Inspectorate office of the Health and Safety Executive.

10. Potatoes should not be fumigated more than once with 2-aminobutane.

EQUIPMENT FOR DETECTING GASEOUS 2-AMINOBUTANE

11. There are several chemical methods of detection but the preferred method is the use of gas detection tubes designed for use with ammonia*. These are small glass tubes filled with silica gel containing a suitable indicator. When the gas is aspirated through the tubes with a bellows attachment** the presence of 2-aminobutane causes a distinct colour change from yellow to blue-violet. The length of colouration along the tube together with the number of aspirations, gives a measure of the air concentration of the fumigant. Tests have shown that the tubes are rather more sensitive to 2-aminobutane than ammonia, and to calculate the concentration of 2-aminobutane, results should be multiplied by 0.75. For example, if the reading indicates a concentration of 20.0 ppm, this should be multiplied by 0.75 which gives 15.0 ppm - the actual concentration of 2-aminobutane.

PROTECTIVE EQUIPMENT

12. Personnel handling the liquid must observe the following precautions. They must wear rubber gloves and eye shields. As the liquid is flammable, operators must not smoke. While there should be no danger from the vapour during handling of the liquid, spillages of 2-aminobutane should be removed by flushing away with plenty of water, and where possible any operations involving the liquid should be carried out in well ventilated places. Should any build up of vapour be detected at any time during the fumigation, and it is necessary to remain in the area, then a respirator fitted with a type CC canister (or other suitable canister) should be worn. Note that canister respirators are not suitable for use in high concentrations or for prolonged periods. However, it should be emphasised that provided correct procedures are followed, the use of 2-aminobutane for the fumigation of potatoes should not result in air concentrations of the fumigant necessitating the use of protective respiratory equipment. Detection of such concentrations in enclosed working areas during application and circulation of the fumigant will, therefore, suggest that the fumigation structure is faulty or that correct procedures are not being followed. Moreover, certain mobile systems are so designed that they will not give rise to high concentrations when operated under normal farm conditions in open stores or other well ventilated stores.

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* Ammonia 5/a tubes manufactured and supplied by Draeger Safety Ltd, Draeger House, Sunnyside Road, Chesham, Bucks HP5 2AR

** Multi gas detector, model 21/31 - Draeger Safety Ltd.

13. Suitable respiratory protective equipment* should be available for all operators taking part in fumigations where there is a possibility of any build up of vapour, and arrangements must be made for its inspection, and servicing if necessary, before any fumigation operations are undertaken.

THE FUMIGATION CHAMBER AND ITS EQUIPMENT

14. As indicated in paragraph 2, the treatment of potatoes must only be carried out in purpose-built fumigation chambers or in buildings specially adapted for the purpose. In some cases fumigation operations in such structures will be covered by the Factories Acts. The approval of the Agriculture Departments must be obtained before any fumigation operations are undertaken. After approval has been given, any alterations to the structure or in the procedures used must be notified to the Agricultural Departments.

15. A satisfactory fumigation structure will normally consist of a gas-tight chamber fitted with a forced draught ventilation system. A vaporiser, capable of vaporising the necessary amount of fumigant within 30-40 minutes, will be attached to the chamber, and if appropriate a series of valves provided so that the vaporised fumigant may be admitted to the chamber, circulated and finally exhausted from the chamber. Chambers, piping, and air ducting must be maintained in a sound condition. Joints should be checked for leaks during operations, and loading doors must have an adequate locking device. To ensure an efficient treatment, it is essential that the air circulation fans are capable of moving a sufficient volume of air through the potatoes being treated, and that the circulation is continued for at least $2\frac{1}{2}$ hours after the fumigant has been introduced. However for larger scale treatments or treatments done under special conditions it may be necessary to modify the time of application and circulation.

FUMIGATION PROCEDURES

16. The fumigation chamber is loaded with potatoes and the vaporiser heater is switched on. (It may be necessary to switch on the vaporiser before loading, depending on the time taken for the vaporiser to reach the operating temperature.) All extraction vents, where fitted, are shut; the doors are closed and also sealed with masking tape or other impervious material if necessary. The required dose 280 ml/tonne ($\frac{1}{2}$ pint/ton) is measured into a container, and when the vaporiser is up to temperature (70°C or above) the circulation fan is switched on. Only when this stage has been reached should the fumigant be introduced into the vaporiser at a predetermined rate, which is dependent on size of installation. Checks must immediately be made for leaks using gas detector and if there are any bad leaks or other faults, introduction of the fumigant into the vaporiser should be stopped and the fan switched off until the fault has been rectified. For detection of small leaks it may be advantageous to continue running the fan but application of the fumigant should be stopped. Introduction of the fumigant into the vaporiser should also be stopped if the recirculation fan fails

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* Suitable respirators and canisters may be obtained from Siebe Gorman Co Ltd., Cwmbran, Gwent, through one of their UK agents.

during application of the fumigant. This is particularly important because the failure could result in the build up of gas concentrations above the explosive limit of 2.1-2.5%. During normal running, because of absorption by tubers, the concentration of vapour in the apparatus rarely exceeds 0.1% - well below the explosive limit.

17. Air is circulated for a period of time after all the fumigant has been applied, and the fan is then switched off. The exhaust vents are opened or where not fitted, the doors opened slightly and the exhaust fan switched on and run until the atmosphere in the chamber is free of 2-aminobutane. In some chambers it is necessary to use a gas detector to check whether the chamber is free of gas because the potatoes are often found to give off a slight odour which may mask the smell of any 2-aminobutane present and it sometimes happens that a similar odour is given off from the walls of the chamber. The extraction fan should be run continuously while unloading the potatoes from the chamber. However, with some mobile systems, the volume of the free space between and around the potatoes is so small that the use of an exhaust fan and detector may be unnecessary.

18. Written instructions listing, in turn, each operation to be followed must be made available to the operator in charge. These instructions will be inspected and form part of the approval of the fumigation plant.

19. At the end of the fumigation certain areas in the potatoes may be damp due to translocation of moisture. This surface dampness can be dissipated by further ventilation. Under certain circumstances and especially in bulk treatments of 20 tonnes or more, moisture can condense in the lower parts of the stack during recirculation, particularly if the potatoes were lifted under wet conditions, so that it may be necessary to ventilate to atmosphere for a period after the fumigation. However, good storage practice, keeping the potatoes cool and well ventilated should avoid problems. No reduction in efficiency of the treatment will occur because of subsequent ventilation.

STORAGE OF 2-AMINOBUTANE

20. 2-aminobutane is highly flammable (flash point - 19.5°C). All containers should be clearly labelled and stored in a cool well-ventilated place under lock and key.

FIRST AID MEASURES

IF THE VAPOUR HAS BEEN INHALED; remove from exposure, rest and keep warm; obtain medical attention.

AFFECTED EYES; irrigate thoroughly with water and in severe cases or where splashing has occurred, obtain medical attention.

IF SPLASHED ON THE SKIN; wash immediately.

IF SPLASHED ON CLOTHING; remove contaminated clothing and wash the skin where the chemical has soaked through; wash the clothing before re-use.

IF SWALLOWED; wash mouth out thoroughly with water and give plenty of water to drink; obtain medical attention.

FURTHER ADVICE

21. Advice on the use of 2-aminobutane as a fumigant to protect potatoes from fungal attack whilst in store may be obtained from:

The Department of Agriculture and Fisheries for Scotland
Agricultural Scientific Services
East Craigs
Edinburgh EH12 8NJ (Tel. 031 334 0355)

The Ministry of Agriculture, Fisheries and Food
Pest Infestation Control Laboratory
London Road
Slough, Berks SL3 7HJ (Tel. 0753 34626)

The Ministry of Agriculture, Fisheries and Food
Plant Pathology Laboratory
Hatching Green
Harpendon, Herts (Tel. 058 27 5241)

The Health and Safety Executive
HM Factory Inspectorate
Baynards House
1 Chepstow Place
London W2 4TF (Tel. 012 29 3456)

The Department of Agriculture for Northern Ireland
Dundonald House
Upper Newtownards Road
Belfast BT4 3SB (Tel. 0232 650111)

Recs/1292
Issued: 30.9.77
Replaces Recs/944

CHEMICAL COMPOUNDS USED AS PESTICIDES

Recommendations for Safe Use in the United Kingdom

2-AMINOBUTANE

A fungicide

(Agricultural and Horticultural Use)

Protection of Operators

1. These recommendations are to be read in conjunction with the advisory leaflet entitled 'The Safe Use of 2-aminobutane for fumigation of Potatoes', obtainable free from the suppliers of the chemical or Agricultural Departments.

2. The fumigation of potatoes with 2-aminobutane may be conducted only under licence from the National Research Development Corporation and the process used must be that cleared by Government Departments under the terms of the Pesticides Safety Precautions Scheme.

3. Other precautions which should be taken and which should appear on the label are:

   FLAMMABLE – Keep away from heat, sparks or open flames.

   2-aminobutane is irritating to the skin and eyes and its vapour is dangerous if breathed. Loosen closure cautiously before opening.
   WEAR PROTECTIVE GLOVES AND FACESHIELD when handling the chemical.
   WASH SPLASHES from skin or eyes immediately and call a doctor.
   AVOID ALL CONTACT BY MOUTH.
   AVOID BREATHING VAPOUR ENSURE EFFICIENT VENTILATION THROUGHOUT WORK AREAS.
   WASH PROTECTIVE CLOTHING after use, especially the inside of gloves.
   WASH HANDS AND EXPOSED SKIN before meals and after work.

Protection of Consumers

Cleared use of 2-aminobutane.

For the fumigation of seed potatoes.

4. The consumption of crops grown from potatoes treated with 2-aminobutane should not present a hazard to consumers.

Protection of Livestock, Wildlife and others

5. As a general precautionary measure the following advice which should appear on the label, should be observed:

DO NOT CONTAMINATE PONDS, WATERS AND DITCHES with chemical or used container.

STORE IN ORIGINAL CONTAINER, tightly closed, in a cool place under lock and key.

EMPTY CONTAINER COMPLETELY and dispose of safely.

Ministry of Agriculture, Fisheries and Food
Pesticides Branch
Great Westminster House
Horseferry Road
London SW1P 2AE

PATENT SPECIFICATION (11) 1268490

1268490

NO DRAWINGS

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(72) Inventors DENNIS COULTHARD GRAHAM and GEORGE ALFRED HAMILTON

(54) METHOD OF CONTROLLING FUNGAL DISEASES IN POTATO TUBERS

(71) We, NATIONAL RESEARCH DEVELOPMENT CORPORATION, a British Corporation established by Statute, of Kingsgate House, 66/74 Victoria Street, London, S.W.1, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of controlling fungal diseases in potato tubers.

Fungal diseases of potato tubers are very serious problems in the production of both seed and ware potatoes, and result in considerable losses in storage every year. The most serious disease is gangrene, a rot of tuber flesh, caused by species of *Phoma*, and experience shows that many widely grown varieties are susceptible. Another important disease is skin spot, caused by the fungus *Oospora pustulans*, although in this case the organism does not rot the tuber, but instead causes the formation of pimple-like pustules on the tuber surface, and, more importantly, causes death of eyes which affects sprouting. Both these diseases are particularly troublesome in the seed trade, because they cannot be seen at lifting, and usually take some time to appear in storage, so that the producer can sell apparently healthy seed which becomes diseased later when in the hands of the buyer. Because of the prolonged incubation period, both skin spot and gangrene are often referred to as "latent diseases".

Control of these diseases has proved difficult and, up till now, satisfactory control has been achieved only by disinfecting washed tubers with solutions of organo-mercury compounds at lifting time. This process has been adopted commercially and has been described in the scientific literature, but it has several difficulties and drawbacks, including the toxic hazard arising from the use of mercurials, and although it has been in use for a considerable period, so far it has made only limited impact on the seed trade in general.

Treatment of tubers with gaseous fungicides has not been investigated much, although treatment with formaldehyde gas has been tried unsuccessfully. Nevertheless, fumigation of tubers has attractive possibilities, since gases could easily be introduced into bulks of stored tubers.

Preliminary experiments were done by treating pure cultures of gangrene and skin spot fungi with gases including methyl bromide, propargyl bromide and chloropicrin, but for various reasons these substances were found unsuitable. Gaseous *sec*-butylamine was also tested in the same way but poor fungicidal activity was observed. However, treatment of infected potatoes in bulk by means of this amine was found to give a surprisingly high fungicidal effect.

According to the present invention, therefore, there is provided a method of controlling fungal diseases in potato tubers comprising treating the tubers with an alkyl amine or a salt thereof in an amount sufficient to kill the fungus or inhibit fungal growth.

The amine may be in the form of a salt thereof, and may be applied to the vegetable in solution, or in a solid, liquid or gaseous phase.

Preferably the amine is gaseous *sec*-butylamine; it is found, unexpectedly, that by fumigating infected potato tubers with this gas at various dosages, very satisfactory control of the diseases is obtained.

The present invention also comprises potato tubers whenever treated by the method of the present invention.

An embodiment of the present invention will now be described by way of example.

Infected potato tubers were lifted from the ground and within three days of lifting were fumigated, at various dosages, with gaseous *sec*-butylamine. The fumigation was effected in a fumigation chamber, and the following results were obtained:—

[*Price 25p*]

EFFECT OF TREATMENT OF 1 HUNDREDWEIGHT OF TUBERS WITH 2-AMINOBUTANE ON INCIDENCE OF GANGRENE

Variety	Dosage (mg/kg) 2 - aminobutane	No. Tubers Examined	% Gangrene
Majestic Stock 1	117	223	4.9
	139	227	4.8
	85	229	6.1
	control (boxed)	195	33.3
Redskin	142	162	2.5
	142	127	0.8
	control (boxed)	171	14.0
Majestic Stock 2	142	172	4.7
	142	177	6.7
	control (boxed)	171	18.1
King Edward Stock 1	143	278	2.5
	357	288	4.2
	control (boxed)	259	25.9
King Edward Stock 2	59	288	0.8
	118	381	1.3
	119	335	0.0
	85	373	0.0
	control (boxed)	399	3.8

EFFECT OF TREATMENT OF 5 TON BULKS OF TUBERS WITH SEC-BUTYLAMINE ON INCIDENCE OF GANGRENE (DOSAGE 200 mg/kg)

Variety	Treatment	No. Tubers Examined	% Gangrene
Majestic	Treated	1328	0.2
	Control	1376	4.1
King Edward	Treated	1605	0.1
	Control	1458	5.8
Redskin	Treated	567	0.7
	Control	552	88.6

EFFECT OF TREATMENT OF 5 TON BULKS OF TUBERS WITH SEC-BUTYLAMINE ON INCIDENCE OF SKIN SPOT (DOSAGE 200 mg/kg)

Variety	Treatment	Percentage Tubers Affected in Each Category							
		Eye Infection Score			Skin Cover Score				
		Free	Some	All	None	Trace	Slight	Mod.	Sev.
King Edward	Treated	100	0	0	100	0	0	0	0
	Control	13	56	31	0	20	70	9	1
Majestic	Treated	100	0	0	98	2	0	0	0
	Control	14	76	10	2	32	46	18	2

EFFECT OF 2-AMINOBUTANE TREATMENT ON INCIDENCE OF SKIN SPOT ON KING EDWARD (1 HUNDREDWEIGHT OF TUBERS USED IN EACH CASE)

Treatment Dosage (mg/kg)	Percentage Tubers Affected in Each Category							
	Eye Infection Score			Skin Cover Score				
	Free	Some	All	None	Trace	Slight	Mod.	Severe
Stock 1								
118	76	24	0	66	26	8	0	0
119	66	34	0	40	52	8	0	0
70	84	16	0	68	30	2	0	0
Control boxed	24	48	28	10	28	58	4	0
Control bagged	2	28	70	0	0	28	36	36
Stock 2								
143	98	2	0	92	6	2	0	0
357	100	0	0	98	2	0	0	0
Control boxed	66	24	10	30	36	20	14	0
Control bagged	10	28	62	2	4	28	24	42

Skin spot assessment after Boyd, A. E. W., Ann. appl. Biol. 45, 284—292, 1957.

An examination of treated tubers just before planting showed no deleterious effects on the tuber skin or on sprouting. Emergence and growth of stems in the field were normal.

On the basis of these results, it can be seen that treatment of potato tubers with gaseous *sec*-butylamine within three days of lifting controls the fungal diseases gangrene (Phoma spp.) and skin spot (Oospora pustulans), particularly favourable results being obtained within the dosage range 100—400 mg. *sec*-butylamine per kg. of potatoes. A preferred range of dosage is from 150 to 250 mgm/kg, but the dosage with *sec*-butylamine may be at least 500 mgm/kg and even as high as 1000 mgm/kg with safety.

Care may need to be taken regarding residues of alkyl amine in treated potato tubers intended for human consumption. Although the present invention has been described with particular reference to the use of *sec*-butylamine on potato tubers, other fungicidally-active amines may be used, for example methylamine, tert-butylamine, isopropylamine and tert-octylamine.

Attention is directed to "The Preservatives in Food Regulations, 1962" in respect of the use of alkyl amines and salts thereof in the treatment of potato tubers intended for food.

WHAT WE CLAIM IS:—

1. A method of controlling fungal diseases in potato tubers comprising treating the tubers with an alkyl amine or salt thereof in an amount sufficient to kill the fungus or inhibit fungal growth.

2. A method according to claim 1, wherein the dosage range is 100—1000 mg alkylamine per kg of tubers.

3. A method according to claim 1 or claim 2 wherein the alkyl amine is applied to the tuber in gaseous phase.

4. A method according to any one of the preceding claims, wherein the alkyl amine is *sec*-butylamine.

5. A method according to claim 4, wherein the *sec*-butylamine is applied in a dosage within the range 100 to 400 mgm/kg.

6. A method according to claim 1 of treating fungal diseases in potato tubers, substantially as hereinbefore described.

7. Potato tubers whenever treated by the method according to any one of claims 1 to 6.

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Summary of results of experiments on the control of the potato tuber fungal diseases gangrene, dry rot and skin spot with various chemical substances, 1966-80.

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Key words: potato, fungicides, tubers, chemical residues, disease, control.

Summary

Over the years 1966-80, experiments were done comparing the effectiveness of a number of known fungicides and certain other chemical substances for the control of the postharvest potato tuber diseases gangrene (Phoma exigua var. foveata), dry rot (Fusarium solani var. caeruleum), and skin spot (Polyscytalum (Oospora) pustulans). Generally good control of gangrene and skin spot, was achieved by fumigation with 2-aminobutane (Sec-butylamine) but dry rot was not controlled. Thiabendazole mists and fog, and thiophanate-methyl and imazalil mists did not give as good a control of gangrene as gaseous 2-aminobutane. However a mist formulation of an admixture of thiabendazole and a salt of 2-aminobutane was as effective as gaseous 2-aminobutane against gangrene, but the formulation proved unstable and unsuitable for general use. It is concluded that although none of the materials tested controlled all diseases equally well, there are several active fungicides whose use can be adapted to the particular need and storage facilities of farmers and merchants. In some cases where active fungicides did not control pathogens, failure was traced to too low a chemical residue or poor distribution of the chemical over the tuber surface, suggesting faulty treatment or unsatisfactory methods of application.

Introduction

Studies on the chemical control of certain common postharvest potato tuber diseases in Scotland, especially gangrene (Phoma exigua var. foveata), skin spot (Polyscytalum (Oospora) pustulans) and dry rot Fusarium solani var. caeruleum) have been carried out

over many years at Agricultural Scientific Services. These diseases, especially gangrene and skin spot, remain serious problems for both the seed producer and purchaser, and can cause heavy losses during storage or when affected tubers fail to emerge or produce weak plants in the field.

Since its introduction in the late 1940's, tecnazene (tetrachloronitrobenzene; TCNB) has remained the most widely used chemical in Scotland (Tucker, 1978). However, although this substance controls dry rot, its main use is as a sprout suppressant. From the early 1950's until the late 1960's, substantial quantities of seed tubers were disinfected by dipping in solutions of organomercury compounds, especially methoxyethylmercury chloride (Boyd, 1960; Graham 1960; Boyd and Penna, 1967). Because of the toxicity of mercurials, the persistence of mercury in the environment, difficulties in disposing of spent dipping solutions, and problems of drying wet tubers, alternatives were actively sought, and 2-aminobutane was introduced commercially as a fumigant for control of gangrene and skin spot (Graham et al. 1973). The fungicide thiabendazole, especially when applied as a mist, was found to give control of other tuber diseases as well as skin spot and gangrene (Logan et al. 1975) and its use has also been commercially developed.

During the course of investigations at Agricultural Scientific Services, a number of known fungicides and other chemical substances have been tested for their effectiveness against tuber diseases, and this paper summarises the results of experiments done over the period 1966-80. The purpose of the experiments was to try to find other active substances, and also to gain an intimate knowledge of the activity of commercially available products in the general context of the Department of Agriculture and Fisheries for Scotland responsibilities for production of healthy seed potatoes. The following substances were tested: propargyl bromide, chloropicrin, ammonia, dibromotetrachloroethane, dichlorophen, cyclohexylamine, thiophanate-methyl and imazalil. In many experiments, their effectiveness was compared with 2-aminobutane and thiabendazole.

Materials and Methods

Harvesting and handling potato tubers

Tubers of various cultivars were received in bulk (usually 250 kg to 500 kg), from farms known from experience to be likely to produce tubers carrying relatively high levels of fungal inoculum, especially of gangrene and skin spot. The tubers were graded over a reciprocating riddle within a day or two of receipt, treated, and then stored in potato trays in a cool store fitted with thermostatically controlled heaters to ensure that temperatures did not fall below 2.0°C.

The handling treatments corresponded as closely as possible with ordinary commercial practice used with high grade seed , and tubers were never inoculated or damaged deliberately to enhance disease levels.

Application of chemicals

Volatile substances were applied in the gaseous phase in one or other of the fumigation chambers described by Graham et al. (1973); ammonia was applied in the same way. Fogs were generated with a "Swingfog" machine (Jaydon Engineering) Co Ltd, England); the fogs were introduced into fumigation chambers through the ducts, and recirculated for 30 minutes to distribute the aerosol, then leaving for another 3-4h to allow deposition to complete. Mists were applied using the "Newforge Potato Mister" (BMC Metal Products, Northern Ireland) or the Mantis Mafex (Mantis GmbH, Hamburg, West Germany). In the case of dichlorophen dip, tubers were immersed for 2 minutes and then dried in trays.

As the time elapsing between harvest and treatment is well known to affect efficiency of control (for example, see Graham et al. 1973) this period was always recorded.

Disease assessments

Tubers were examined for rot diseases in storage on two occasions, first in late January to early February and again in late March to early April.

For skin spot assessment, samples of 50 to 100 tubers were washed and examined in April. For simplicity, the degree of skin spot infection was expressed only as the Surface Infection Index (Boyd, 1957).

Disease diagnosis was based largely on visual symptoms, but in a representative proportion of affected tubers the disease identity checked by culturing fungi on appropriate media. For confirmation of the presence of skin spot, eye plugs were removed, incubated in moist chambers and examined microscopically, according to the method of Hide et al. (1968).

Chemical residue levels

It is notable that comparison of the biological efficiency of chemical treatments reported by different workers using the same substances and ostensibly the same methods of application shows that there can be considerable variations between levels of disease control. A major factor which affects efficiency is the time elapsing between harvest and treatment, as has already been mentioned; on the whole, the longer treatment is delayed after lifting, the poorer the control will be. This is generally thought to arise through suberization of skin beginning after haulm destruction and accelerating after harvest with a consequent decrease in skin permeability to the toxicant. Nevertheless, there have been many cases both in our experience or which have been drawn to our attention, where treatments have been applied soon after harvest and before maturation of the skin has had time to take place, but considerable variations have still occurred. The reasons for this are not clearly understood, but several explanations can be offered, such as differences in the skin structure of different cultivars and/or the depth to which the fungus has penetrated into the tissues before treatment. In turn these differences are affected by the weather conditions and cultural treatments during the growing season.

In earlier work, it was found that in a number of instances where methoxyethylmercury chloride dips had failed to control gangrene satisfactorily, the most likely explanation for ineffectiveness was failure by the users to allow

sufficient time for the mercurial to dissolve before using the dipping solution. In one case analysis of the dipping fluid showed that the concentration of the mercurial in solution was only 5.0 mg/kg instead of the theorietical 120.0 mg/kg, and the residue in the peel only 2.0 mg/kg, or less. More recently in connection with commercial 2-aminobutane fumigations, failures to control gangrene have almost always been traced to too low residue levels for treatment to be effective, indicating faulty applications of chemical.

By analogy, it seemed likely that poor control of fungal pathogens with other chemicals could result from nothing more than inadequate applications giving low and therefore ineffective chemical residue levels. Thus, in the later years of this series of experiments, analyses of treated tubers have been done routinely to see how the dose applied compared with the residues actually achieved.

A survey by Hamilton and Lindsay (1980) showed that uneven and low fungicide residues were commonly present in tubers treated with thiabendazole by farmers. Twenty-three samples had levels of less than 2.0 mg/kg, 10 had between 2.0 to 5.0 mg/kg and 24 had more than 5.0 mg/kg. These tubers were analysed without removal of attached soil.

Residue analytical procedures

Tubers were stored in plastic bags in a cold store at 4°C before analysis, to minimise any loss of residue that might occur by volatilization. Samples consisting of sound quarters from each of at least four tubers of fairly uniform size. Only excessive amounts of attached soil were removed before sub-sampling and analysis. It was necessary to take samples from several tubers because it is known that there can be considerable variations between individual tubers (c.f. Hamilton and Ruthven (1967) for mercury, and Graham et al. (1973), for 2-aminobutane).

The residues were determined by the following methods.

Tecnazene. Peel (about 1 mm in thickness) was dried with anhydrous sodium sulphate, residues extracted with hexane, and determined by gas-liquid chromatography (GLC) using electron-capture detection.

2-aminobutane. Initially residues of 2-aminobutane were determined by GLC (Day et al. 1968) as carried out by Graham et al. (1973), and after 1975 by high performance liquid chromatography (HPLC) (Hunter and Lindsay 1979).

thiabendazole. Macerated whole potato was dried with anhydrous sodium sulphate and thiabendazole extracted with ethyl acetate. Residues were determined by reverse-phase HPLC using fluorescence detection (Hunter 1979).

Imazalil. Imazalil was extracted in the same way as thiabendazole. After liquid-liquid partition clean-up, residues were determined by reverse-phase HPLC using ultra-violet detection (Hunter 1979).

Results

Table 1 gives details of the chemical treatments, period elapsing between harvest and treatment, application methods, dose, and chemical residue levels, where available. Residue data are expressed in terms of whole tubers.

(Table 1 near here)

Table 2 lists the results for control of gangrene, dry rot and skin spot, using different potato cultivars, from 1966 to 1979.

(Table 2 near here)

Propargyl bromide did not control gangrene, but apparently controlled skin spot. Low doses of chloropicrin appeared to give some control of gangrene and skin spot, but were too phytotoxic for use. Both chemicals were unpleasant to handle. Dry rot was absent in the stocks used.

Gaseous ammonia gave some control of gangrene and skin spot, but appeared to increase dry rot. There was marked evidence of phytotoxicity, including severe lenticel pitting and very large dark brown sunken areas where tubers were mechanically damaged. Ammonia has long been known to have fungitoxic properties, and has been used experimentally to control postharvest fungal diseases of various crops (Eckert, 1977). Interestingly, ammonia behaved physically very differently from 2-aminobutane in that it was not rapidly adsorbed, then desorbed and resorbed through a column of potatoes. It passed rapidly through the bulk of the potatoes and was only slowly absorbed by tubers during recirculation (cf. Graham et al. 1973).

Dibromotetrachloroethane (1,2-dibromo - 1,1,2,2-tetrachloroethane, DBTCE) was difficult to vaporise, and appeared to partly decompose, giving white fumes which probably contained hydrogen bromide. The treatment appeared to give some control of dry rot, and it is notable that DBTCE was originally patented for this purpose as long ago as 1958 (formulated as a dust), although it does not appear to have been marketed commercially (U.S. Patent 2853415). There was also some evidence that skin spot was controlled, but not gangrene. It is also noteworthy that DBTCE treatment seemed to

cause a marked increase in tuber blight (Phytophthora infestans); in one experiment 9.5% of treated tubers showed infection, compared with 2.5% in the untreated control. The reason for this is not clear, but the chemical may suppress a natural defence reaction, thus allowing the development of cryptic blight infection into visible symptoms.

Poor results were obtained against all three diseases with dichlorophen fogs and dips, although there was some control of gangrene and skin spot by the dips. Dichlorophen fog was very acrid and unpleasant to use. Cyclohexylamine was relatively easy to vaporise (b.p. 134.5°C) and proved not unpleasant to handle, but did not give adequate levels of control of gangrene or dry rot (there was no skin spot infection in the test material).

Thiabendazole fogs applied as recommended commercially gave poor control of all three diseases, despite the fact that thiabendazole is known to be active against all three fungi. The results are, however, not surprising as the theoretical residue was only 2.2 mg/kg and actual levels were well below this, as shown in table 1. (It is generally considered that the minimum effective dose is 5.0 mg/kg (c.2.0 mg/kg for treatments with the Mantis Mafex), although levels between 15-20 mg/kg are much more desirable.) Thiabendazole mist applied by both the Newforge and the Mantis machines (dispersible powder and flowable formulations) also gave relatively poor control of all three diseases although its effectiveness against dry rot could not be judged accurately because of low disease levels in untreated material. The theoretical dose of 40.0 mg/kg was never approached and residues varied from 2.9-22.8 mg/kg with the Newforge and 11.8-19.5 mg/kg with the Mantis machine. Moreover, the higher levels recorded in 1979 resulted from treatments where application rates were adjusted so that all tuber surfaces received what appeared to be adequate cover. A complicating factor was delay between treatment and harvest since it is known that efficiency of thiabendazole can decrease rapidly after lifting (Boyd, 1977) and the delays may well have mitigated against good control in some cases. Nevertheless, the commercial recommendations state that treatment can be applied up to 14 days after lifting, and it is notable that the treatments in 1978 were done 2, 3,

4 and 6 days after harvest, and in 1979, 1 day after harvest. The Newforge misting apparatus was rather difficult to use - a good deal of the mist sprayed on to the sides of the metal canopy from the spinning disc generator, and liquid fell from the sides of the canopy on to the moving table carrying the tubers. This may partly account for the low residue levels. The Mantis Mafex was easy to use, but deposition was difficult to judge by eye. However, by introducing a fluorescent tracer into the formulation and viewing tubers under ultraviolet light, it was clear that much of the tuber surface had received no chemical at all. This could account for the poor disease control.

Thiophanate-methyl mist failed to give satisfactory control of gangrene and skin spot. Imazalil mist also failed to give good control of gangrene, but there was insufficient dry rot or skin spot infection to judge their efficacy against these diseases. In the case of imazalil, control might be improved if the dose was increased to levels corresponding with the dose of thiabendazole used commercially; low residues (in the region of 3.0 mg/kg) may be part of the reason why the treatment failed to give adequate control.

Good results in controlling gangrene and skin spot were achieved by misting with a formulation of thiabendazole plus a salt of 2-aminobutane, even although residue levels were again well below the theoretical values. Effectiveness was comparable with that given by gaseous 2-aminobutane.

Tecnazene fog was tested mainly to see whether treatment controlled dry rot, but there was little or no infection in the potato stocks used. However treatment did not affect sprouting; one reason probably was the very low residues found soon after treatment, and tray storage would also allow the chemical to vaporise readily from the tubers. Combined treatments with 2-aminobutane gas and tecnazene fog showed that these materials were compatible and did not cause chemical damage or effect sprouting.

Discussion

So far, no completely satisfactory fungicides or combination of fungicides have been found to control all the three major postharvest fungal diseases of potatoes found in Scotland. Problems associated with chemical control of postharvest diseases affecting a wide range of plant material, including potatoes, have been reviewed by Eckert (1977). Thiabendazole potato tuber treatments have been extensively investigated by several workers in the United Kingdom including Logan et al.(1975), Boyd (1977) and Cayley et al. (1979).

Results obtained with thiabendazole treatments illustrate the need for toxicants to have sufficient penetrative action to pass through tuber skin, for even where adequate doses had been applied control was poor. It is thought that, in such cases the fungus was too deep-seated for sufficient toxicant to reach it (as in 1979). The results of the Mantis/Mafex treatments serve to illustrate another factor, namely inadequate distribution of the toxicant over the tuber surface. The continued activity of 2-aminobutane in such cases arises, in part, from its capacity to diffuse through skin to depths where a sufficient concentration is achieved to affect fungal growth (Graham et al, 1976). The material treated in 1979 was lifted late in the year (20 November) thus giving the fungus a longer opportunity to penetrate into and through the skin, but lifting and treating tubers in November is commonplace, especially in the more northerly parts of Scotland.

As mentioned above, mist application of formulations containing a mixture of thiabendazole and a salt of 2-aminobutane have given good results in experiments for control of gangrene and skin spot. However, it seems to be very difficult to formulate a stable mixture and so far no preparation has been produced with a sufficiently long shelf life to be commercially acceptable. Regarding mist treatments where the formulation is diluted with water, moisture levels are increased, which can, in turn, increase the risk of the development of bacterial soft rots, especially if ventilation is restricted and storage temperatures remain high after lifting.

A drawback to use of 2-aminobutane as a fumigant for potato tubers can be its failure to control Fusarium rots, and a search is continuing to find a substance which could be vaporised in admixture with 2-aminobutane to achieve control. Chemicals including chlorobenzene, bromobenzene, 4-bromoisopropylbenzene, 1,2,3,4-tetramethylbenzene, 1,1,1-trichloroethane and benzyl isothiocyanate have been tested in the vapour phase for their effects on growth of pure cultures of Fusarium solani var. caeruleum, but have been rejected for various reasons, including lack of knowledge of the toxicology of some substances. A very considerable amount of costly data must be accumulated to satisfy registration requirements on safety before any new pesticide can be marketed, especially one which might eventually be used on ware (table) potatoes. Since potatoes form a very important part of the staple diet in many countries fungicides for direct application to ware potatoes must be acceptable toxicologically. Many potential potato fumigants are not patentable fungicides, and chemical companies cannot be expected to obtain extensive residue and toxicological data on substances likely to have only very limited applications and having no patent protection.

Nevertheless, despite the inadequacies of products presently available, their value must not be overlooked, and judicious use of postharvest chemical treatments can do a great deal to minimise losses. Individual growers and merchants should adapt the use of fungicides in relation to their individual disease problems, and to their methods of storage. However, studies of residue levels and distribution demonstrate that present methods of fungicide application need improvement to achieve better loading of fungicide on tubers and thus better disease control.

References

BOYD, A. E. W. (1957) Field experiments on potato skin spot disease caused by Oospora pustulans. Ann. Appl. Biol. 45, 284-292.

BOYD, A. E. W. (1960). Fungicidal dipping and other treatments of seed potatoes in Scotland. Eur. Potato J. 3, 137-154.

BOYD, A. E. W. (1977). Control of certain potato tuber diseases by thiabendazole and 2-aminobutane. Proc. Symposium on Problems of Pest and Disease Control in Northern Britain; Scottish Horticultural Research Institute.

BOYD, A. E. W. & R. J. PENNA (1967). Seed tuber disinfection. Proc. 4th Br. Insectic. and Fungic. Conf. 1, 294-302.

CAYLEY, G. R., G. A. HIDE, K. A. LORD, D. J. AUSTIN & A. R. DAVIES (1979). Control of potato storage diseases with thiabendazole. Potato Res. 22, 171-190.

DAY, E. W., F. J. HOLZER, J. B. TEPE, J. W. ECKERT, & M. J. KOLBEZEN (1968). Determination of sec-butylamine residues in fruit. J. Ass. off. analyt. Chem. 51, 39-44.

ECKERT, J. W. (1977). Control of postharvest diseases. In Antifungal Compounds, pp. 270-352, New York, Marcel Dekker Inc.

GRAHAM, D. C. (1960). Control of Rhizoctonia solani on potato by disinfection of tubers with organo-mercury compounds. Eur. Potato J. 3, 80-89.

GRAHAM, D. C., G. A. HAMILTON, C. E. QUINN & A. D. RUTHVEN (1973). Use of 2-aminobutane as a fumigant for control of gangrene, skin spot and silver scurf diseases of potato tubers. Potato Res. 16, 109-125.

GRAHAM, D. C., G. A. HAMILTON, C. E. QUINN & A. D. RUTHVEN (1976). Control of potato gangrene by fumigation of tubers with 2-aminobutane after periods of storage. Potato Res. 18, 410-415.

HAMILTON, G. A. & D. A. LINDSAY (1980). Unpublished data.

HAMILTON, G. A. & A. D. RUTHVEN (1967). Residual mercury contents of seed potatoes treated with organo-mercury disinfectant solutions. J. Sci. Fd. Agric. 18, 558-563

HIDE, G. A., J. M. HIRST & G. A. SALT (1968). Methods of measuring the prevalence of pathogenic fungi on potato tubers. Ann. Appl. Biol. 62, 309-318.

HUNTER, K. (1979). Private communication.

HUNTER, K. & D. A. LINDSAY (1979). Private communication.

LOGAN, C., R. B. COPELAND & G. LITTLE (1975). Potato gangrene control by ultra low volume sprays of thiabendazole. Ann. Appl. Biol. 80, 199-204.

TUCKER, G. G. (1978). Survey of pesticide usage on potatoes stored from the 1975 Scottish Crop. The Seed Potato. 18, 17-22.

SUMMARY FOR TRANSLATION

From 1966 to 1980, experiments were done to assess the effectiveness of a number of known fungicides and certain other chemical substances (mainly organo-halogen compounds) for control of the postharvest potato tuber diseases gangrene (Phoma exigua var. foveata), dry rot (Fusarium solani var. caerulcum) and skin spot (Polyscytalum (Oospora) pustulans).

Table 1 gives details of the chemical treatments, period elapsing between harvest and treatment, application methods, dose, and chemical residues where appropriate (residue data are expressed in terms of whole tubers). Table 2 lists the results for control of gangrene, dry rot and skin spot using different potato cultivars. Handling and treatments followed commercial practice as far as possible.

Regarding gangrene and skin spot, generally good control was achieved by fumigation with 2-aminobutane, but dry rot was not controlled. Dichlorophen dips and fog were ineffective. Thiabendazole mists and fog, and thiophanate-methyl and imazalil mists did not, in general, give as good a control as gaseous 2-aminobutane. It is suggested that this was due variously to too low residues, inability of the toxicants to diffuse through tuber skin to reach the fungi when they had become deep-seated, and inadequate distribution over the tuber surface. A mist formulation of an admixture of thiabendazole and a salt of 2-aminobutane was very effective against gangrene, but the formulation proved unstable and could not be prepared for commercial use. Several volatile organo-healogen and two other compounds were tested for control of dry rot fungus, with a view to mixing with 2-aminobutane, but were rejected for various reasons, including lack of knowledge of the toxicology of some substances.

It is concluded that although there is no chemical product that controls all three diseases well, there are several active fungicides whose use can be adapted to particular needs and storage facilities of farmers and merchants. There is considerable scope for improving methods of application of mist formulations to achieve higher residue levels and better distribution of the chemicals over the tuber surface.

Table 1. Chemical treatments of different potato varieties during 1974–1979, method of application, dose applied and chemical residue level.

Cultivar[1]	Date of harvest[2]	Days elapsed between harvest and treatment[3]	Chemical and method of application[4]	Dose[5] (mg/kg)	Residue level[6] (mg/kg)
Redskin	17/10/74	5	thiabendazole fog[7]	2.18	0.31
King Edward	7/10/74	15	thiabendazole fog	2.18	0.22
Pentland Dell	15/10/74	7	thiabendazole fog	2.18	0.20
King Edward	15/10/75	15	thiabendazole mist[8] N*	40	5.75
Désirée	20/10/75	11	thiabendazole mist N	40	2.9
Pentland Crown	18/11/77	3	thiabendazole mist N	40	17.9
		3	thiabendazole + 2-aminobutane mist N	40 + 200	12.6 + 67.8
		3	thiophanate-methyl mist N	80	–
		3	2-aminobutane gas[9]	200	66.9
Record	27/10/77	25	thiabendazole mist N	40	13.3
		25	thiabendazole + 2-aminobutane mist N	40 + 200	11.5 + 58.4
		25	thiophanate-methyl mist N	80	–
		25	2-aminobutane gas	200	35.4
Pentland Crown	14/10/78	12	tecnazene fog	18	0.03
		12	thiabendazole mist N	40	7.9
		12	imazalil mist N	8	1.5
		12	2-aminobutane gas	200	36.0
		12	2-aminobutane gas + tecnazene fog	200 + 18	68.8 + 0.10
Majestic	19/10/78	6	tecnazene fog	18	0.06
		6	thiabendazole mist N	40	15.4
		6	imazalil mist N	8	1.1
		6	2-aminobutane gas	200	69.4
		6	2-aminobutane gas + tecnazene fog	200 + 18	38.2 + 0.10
Redskin	22/10/78	4	tecnazene fog	18	0.05
		4	thiabendazole mist N	40	6.9
		4	imazalil mist N	8	1.9
		4	2-aminobutane gas	200	38.6
		4	2-aminobutane gas + tecnazene fog	200 + 18	32.0 + 0.08
Kerrs Pink	23/10/78	3	tecnazene fog	18	0.03
		3	thiabendazole mist N	40	6.8
		3	imazalil mist N	8	2.5
		3	2-aminobutane gas	200	27.0
		3	2-aminobutane gas + tecnazene fog	200 + 18	94.0 + 0.12
Record	24/10/78	2	tecnazene fog	18	0.08
		2	thiabendazole mist N	40	10.2
		2	imazalil mist N	8	1.8
		2	2-aminobutane gas	200	57.4
		2	2-aminobutane gas + tecnazene fog	200 + 18	42.4 + 0.04
Pentland Crown	20/11/79	1	thiabendazole mist N	40	11.3
		1	thiabendazole mist M	40	15.2
		1	imazalil mist N	8	3.0
		6	2-aminobutane gas	200	68.6
Record	20/11/79	1	thiabendazole mist N	40	22.8
		1	thiabendazole mist M	40	11.8
		1	imazalil mist N	8	3.9
		6	2-aminobutane gas	200	44.8
Maris Piper	20/11/79	1	thiabendazole mist N	40	17.1
		1	thiabendazole mist M	40	19.5
		1	imazalil mist N	8	3.4
		6	2-aminobutane gas	200	60.0

* N = Misting by Newforge Mister – *Gespritzt durch Newforge Mister – Pulvérisé avec Newforge Mister.*
M = Treatment with Mantis Mafex – *Behandlung durch Mantis Mafex – Traitement avec Mantis Mafex.*

Table 2. Results of experiments comparing levels of disease control by different chemical treatments, given in Table 1.

Cultivar[1]	Chemical treatment[2]	Number of tubers examined[3]	Gangrene[4] (%)	Dry rot[5] (%)	Skin spot surface infection index[6]
Redskin	thiabendazole fog[7]	446	0.7	63.2	0
	nil	422	2.4	69.0	0
King Edward	thiabendazole fog	679	4.0	0.6	3.90
	nil	663	4.2	0.6	5.28
Pentland Dell	thiabendazole fog	579	1.6	1.2	0
	nil	601	2.2	2.2	0
King Edward	thiabendazole mist[8] N*	661	6.7	0.6	2.62
	nil	816	6.7	0.6	3.20
Désirée	thiabendazole mist N	601	4.0	0.3	0
	nil	720	6.1	0.3	0
Pentland Crown	thiabendazole mist N	687	51.7	0	0
	thiabendazole + 2-aminobutane mist N	777	11.3	0	0
	thiophanate-methyl mist N	710	41.0	0	0
	2-aminobutane gas[9]	608	13.8	0	0
	nil	637	83.7	0	0
Record	thiabendazole mist N	840	13.9	0	1.21
	thiabendazole + 2-aminobutane mist N	860	5.7	0.1	0
	thiophanate-methyl mist N	774	6.9	0.4	0.16
	2-aminobutane gas	889	4.6	0.5	0
	nil	907	18.3	0.1	1.79
Pentland Crown	tecnazene fog	855	20.5	0	0
	thiabendazole mist N	955	18.7	0	0
	imazalil mist N	814	16.7	0	0
	2-aminobutane gas	812	4.2	0	0
	2-aminobutane gas + tecnazene fog	972	2.3	0	0
	nil	941	22.0	0	0
Majestic	tecnazene fog	840	6.9	0.2	0
	thiabendazole mist N	782	5.4	0	0
	imazalil mist N	716	4.2	0	0
	2-aminobutane gas	806	1.9	0.3	0
	2-aminobutane gas + tecnazene fog	912	1.1	0.8	0
	nil	875	6.3	0	0
Redskin	tecnazene fog	997	5.7	0.3	0
	thiabendazole mist N	912	6.5	0	0
	imazalil mist N	803	7.9	0.1	0
	2-aminobutane gas	909	1.3	0	0
	2-aminobutane gas + tecnazene fog	871	1.8	0.3	0
	nil	1018	13.2	0.4	0
Kerrs Pink	tecnazene fog	798	2.7	0	0
	thiabendazole mist N	757	1.5	0	0
	imazalil mist N	600	2.2	0	0
	2-aminobutane gas	801	0.4	0	0
	2-aminobutane gas + tecnazene fog	783	0.3	0	0
	nil	808	2.9	0	0
Record	tecnazene fog	889	3.4	0	0
	thiabendazole mist N	778	3.0	0	0
	imazalil mist N	775	3.2	0	0
	2-aminobutane gas	789	1.1	0	0
	2-aminobutane gas + tecnazene fog	819	0.9	0	0
	nil	740	8.0	0	0
Pentland Crown	thiabendazole mist N	852	38.3	0	0
	thiabendazole mist M*	765	53.5	0	0
	imazalil mist N	766	25.3	0	0
	2-aminobutane gas	726	9.1	0	0
	nil	773	44.3	0	0
Record	thiabendazole mist N	965	11.1	0	0
	thiabendazole mist M	993	17.7	0	0
	imazalil mist N	1059	8.0	0	0
	2-aminobutane gas	861	2.1	0	0
	nil	808	16.7	0	0
Maris Piper	thiabendazole mist N	713	37.8	0	0
	thiabendazole mist M	606	38.0	0	0
	imazalil mist N	717	18.7	0	0
	2-aminobutane gas	778	3.3	0	0
	nil	526	37.8	0	0

* See Table 1 – *Siehe Tabelle 1 - Voir tableau 1.*

ADDENDUM: STUDY OF THE FORMATION OF DROPLETS WHEN SIMULATED RAINDROPS FALL ON BLACKLEG INFECTED STEMS USING HIGH SPEED PHOTOGRAPHY

When raindrops fall in to relatively deep water, the impact causes the formation of a coronet-shaped upward surge of drops which break up into smaller drops, some of which are ballistic and fall back into the water, while others are very tiny and form aerosols. The mouth of the crater formed on impact is closed over to form a bubble. At the same time the fluid collapses back towards the point of impact, and the rebound causes the formation of a central column of liquid (the Rayleigh jet) which may burst the bubble, or the bubble may burst before the central column penetrates it. Bubble bursts also generate tiny drops which form aerosols.

Regarding the generation of aerosols by raindrop impaction on rotting potato stems, the circumstances are different in that drops would fall on soft rotted tissue, which would not behave in the same way as water. The circumstances were considered to resemble the situation where drops fall on to a thin liquid film. This film would form from the water derived from the rotted stem, and very quickly after drops began to fall, from the drops themselves. Experiments show that there is no bubble formation and no Rayleigh jet. The surface film is forced outwards by the drop and surface tension forces cause the formation of a coronet-shaped structure from which droplets are released. The phenomena are well described by Gregory in "The Microbiology of the Atmosphere" 2nd edition, 1971, pp 57-70, Leonard Hill.

With rotted potato stems, Graham, Quinn and Sells (1977) suggested that the aerosols could be generated by both the formation of the coronet-shaped upward surge, and the bursting of bubbles. However, to see what actually happens,

opportunity was taken to study impact of 5 mm diam. drops falling at 94% terminal velocity, at a rate of 180 drops/min. using high-speed photography. The drops were aimed on to one or more potato stems inoculated with E. atroseptica about 48 hr previously, and which were well rotted. The camera used was a Hadland high speed, John Hadland Photographic Instrumentation Ltd) operating at 1000 exposures/sec.

When drops started, a coronet-shaped surge formed, many droplets streaming upwards from the ring, much as described for impact on a thin liquid film. Within a short time - about 2 min., or sometimes even less, the rotted tissue was broken up, and impacts took place on the broken plant material, soil and water mixture. When this happened the formation of droplets became more complex. The coronet form became distorted, and, in some cases long sausage-shaped rather coarse filaments streamed outwards and upwards, these usually breaking up into droplets, especially from the ends of the filaments. Moreover soil had been taken up into many droplets because the droplets were plainly darkened by the soil particles in them. These phenomena continued for up to 5 min., when bombardment was stopped. It was especially notable that no bubbles were observed. The different stages are illustrated in figs. 16-20.

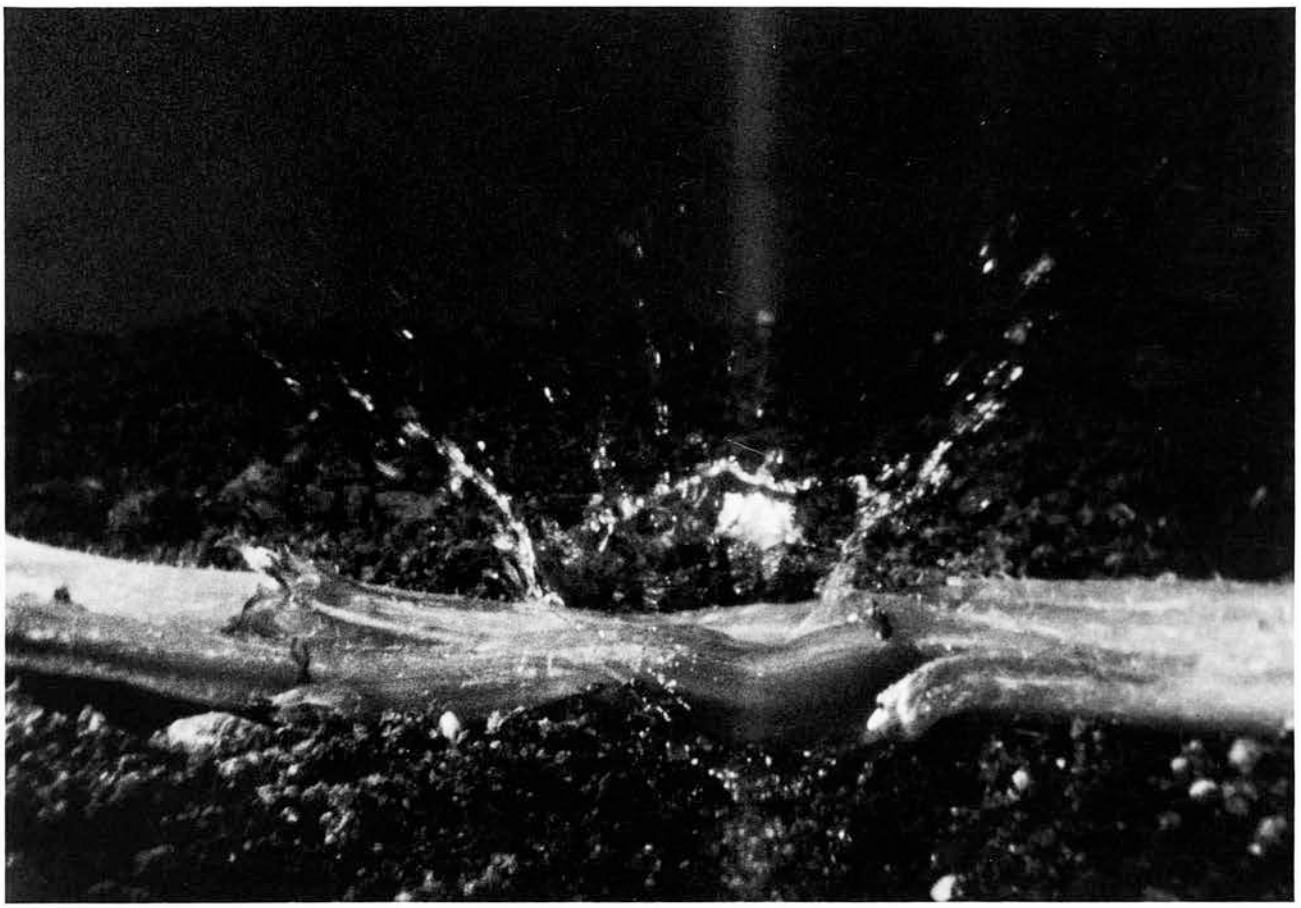

Fig. 16. Photograph of simulated raindrop impaction on rotted potato stems showing processes leading to formation of bacterial aerosols. Coronet shaped upward surge at start of simulated rainfall.

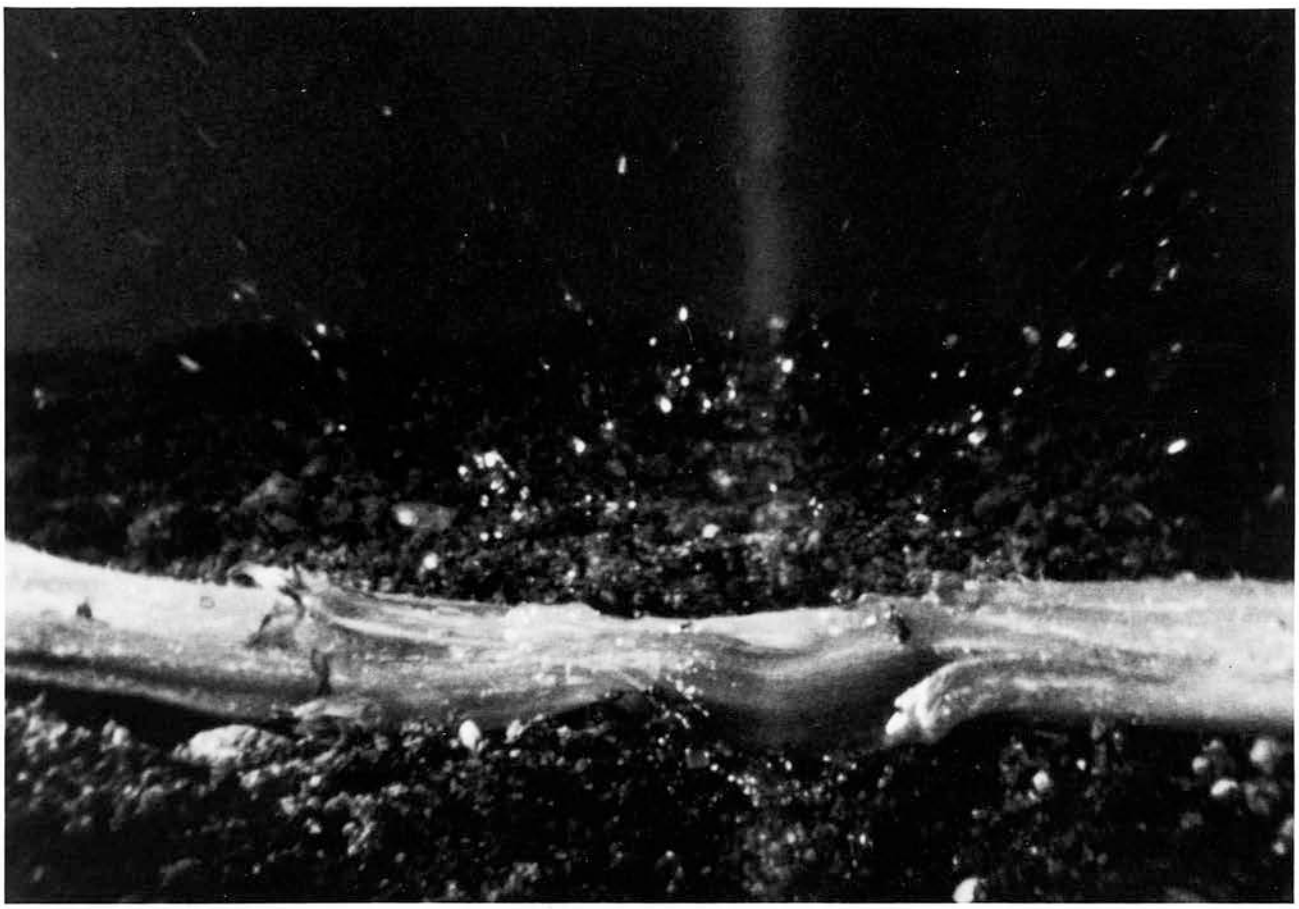

Fig. 17. Photograph of simulated raindrop impaction on rotted potato stems showing processes leading to formation of bacterial aerosols. Coronet collapsed - droplets continuing to disperse.

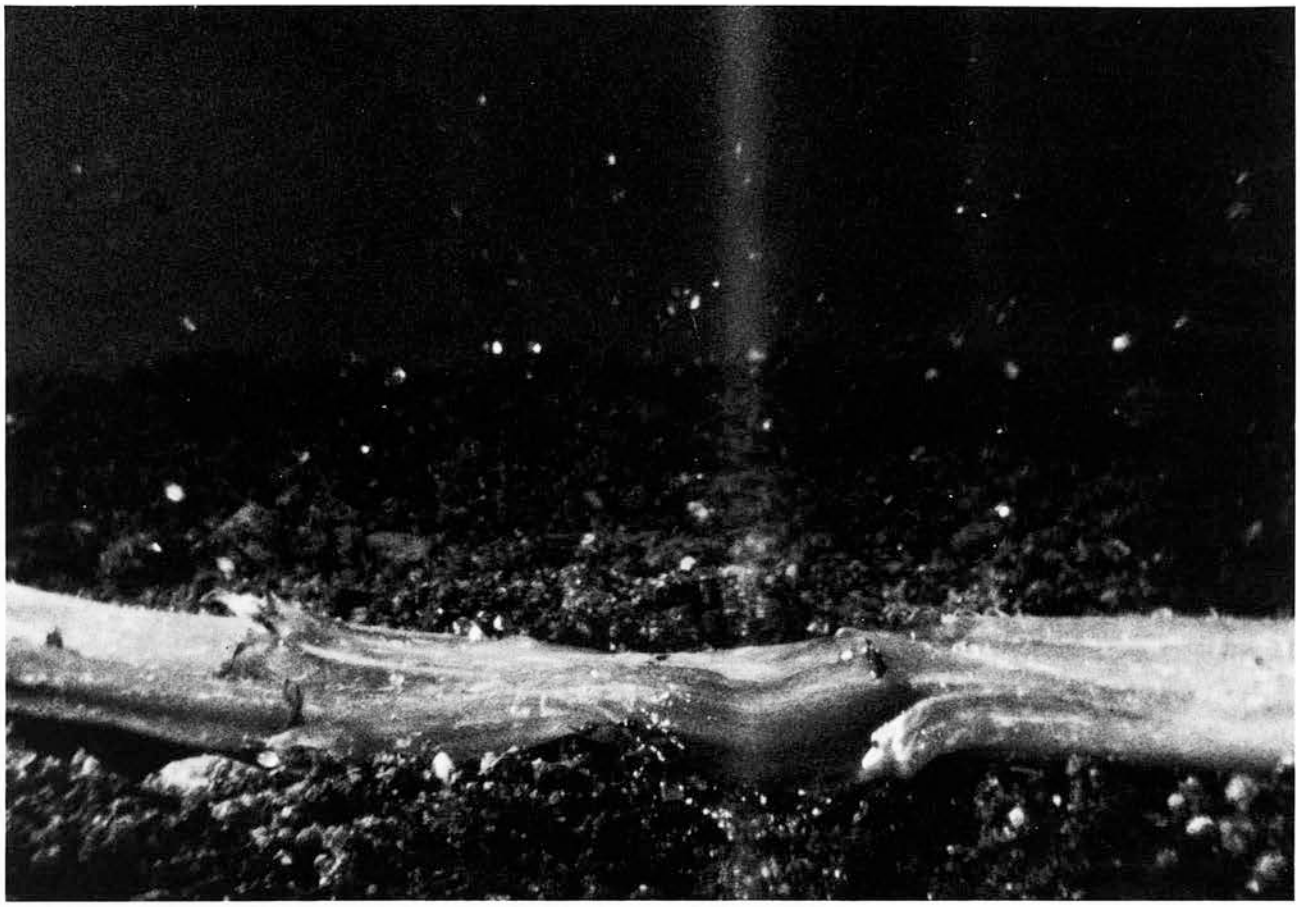

Fig. 18. Photograph of simulated raindrop impaction on rotted potato stems showing processes leading to formation of bacterial aerosols. Further dispersal of drops.

Fig. 19. Photograph of simulated raindrop impaction on rotted potato stems showing processes leading to formation of bacterial aerosols. Later stage, about 3 min. after start of rainfall. No coronet formed. Long "sausage shaped" streamer.

Fig. 20. Photograph of simulated raindrop impaction on rotted potato stems showing processes leading to formation of bacterial aerosols. About 5 min. after start of rainfall. Typical "sausage" streamer.